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"SULFITING AGENTS"

A Monograph Reviewing the Following Substances: Sulfur Dioxide, Sodium Bisulfite, Sodium Metabisulfite, Sodium Sulfite, Potassium Bisulfite, Potassium Metabisulfite and Potassium Sulfite

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SUMMARY

Host response to the various "sulfiting" agents under review (sulfur dioxide, bisulfites, metabisulfites and sulfites) is largely uniform and dependent on the SO_2 equivalency of the moiety administered. Moreover, the systemic effects found are said to be unaffected by portal of entry constraints (44).

Absorption and Distribution Studies

Approximately half of the dietary sodium metabisulfite in rats was absorbed by the gastrointestinal tract and half had apparently already been oxidized before absorption (19), specifically by the small gut.

Sulfur dioxide (SO_2) becomes hydrated directly on contact with body fluids (55, 115). Therefore, it is likely that hydrated SO_2, as fluids in general, escapes significant physical contact with saliva and its modest oxidative potential. Gastric oxidation must also be negligible, since sulfite autoxidation is pH-independent only between 8.8 and 8.2 [Joslyn, M. A. and Braveman, J. B. S. Adv. Food Res. 5, 97 (1954)], an achlorhydric range uncommon for more than 95% of the population. Moreover, the rate of sulfite oxidation becomes negligible between pH 5.9 and 3.2. It would follow that the small gut, especially the duodenum, is the only oxidative site for the non-resorbed moiety. The fact that duodenal juice is relatively alkaline and that sulfate is resorbed only with difficulty is supportive. The duodenal sulfate would be subsequently carried by the systemic circulation for renal clearance.

Resorbed sulfite would pass to the liver via the portal circulation. Striking evidence that organ systems possess sulfite oxidation potential was recently described for a human infant with congenital sulfite oxidase deficiency (94). No inorganic sulfate was detectable in urine before exitus, but there were elevated amounts of sulfite (116). Assay of labeled sulfur distribution in SO_2 inhalation studies found a spleen:kidney:brain:liver localization ratio of 1:1:2:15, which pattern was followed independently of portal of entry (13).

If complete oxidation fails in the liver, the anions would reach the tissues via the systemic circulation. There the serum proteins were found to protect bisulfite from oxidation [Seitz, W. Klin. Wschr. 21 (43), 955 (1942)] in *in vitro* studies if the oxygen increments were small. This was interpreted purely as a pH effect. Greater amounts of oxygen introduced a reversal effect leading to accelerated bisulfite oxidation. This effect was attributed to surface activity. It was found that most of the reactivity in a sulfite-plasma (rabbit) system was non-dialyzable and not undergoing rapid oxidation. In human serum a steady-state was reached in about 1 hour with 80% of the sulfite free, a level which remained constant for up to 3 hours (57). This would be equivalent to the *in vivo* result where sulfite oxidation does not occur at a significant rate due to the presence in mammalian tissue fluid of compounds intercepting free radical chain reactions and the relative absence of free metal ions capable of catalyzing sulfite oxidation (61,50). Serum prophylaxis in SO_2 inhalation studies has been related to the formation of S-sulfonate groups in sulfitolysis of disulfide bonds (57). Evidence that accumulating SO_2, or so-called "cyanolytic sulfite" from cyanide attack on S-sulfonate functions, was more abundant in plasma than serum tends to confirm the sulfitolysis reaction, since plasma fibrinogen would have a higher level of available disulfide bonding. The reaction attained equilibrium at almost complete S-sulfonation or sulfite oxidation. It is also of interest that a zwitterionic sulfonate was proposed as a reaction product between sodium sulfite or bisulfite and epinephrine (121).

There is a reduction in SO_2 concentration in peripheral airways because of high solubility in body fluids. Nevertheless, labeled sulfur in SO_2 aeration studies on dogs have confirmed rapid removal of 90% of the gas from an air admixture and retention by pharnyx, trachea, lungs and hilar lymph (13) in significant deposits. Labeled sulfur could be retained in lungs up to 7 days after acute exposure (40 min) at modest concentrations (33 ppm). After slow release from respiratory tissues via pulmonary and systemic transit, the only organs failing to exhibit detectable label were the adrenals, thyroid, skin, muscle and bladder.

Excretion

The first reported study of sulfurous acid elimination was based on a feeding study in dogs [Sonntag, G. Arb. Kaiserl. Gesundheitsamt 21, 285 (1904)]. After 4-day sodium sulfite diets, which led to vomiting at the 6000mg level, only a fraction (3-5%) could be recovered in urine; the recovery was independent of dose ingested. Lower doses of a hydroxyethane sulfonic acid, which induced diarrhea, also terminated in low sulfite harvest. It was concluded that both agents were excreted principally as sulfate. The earliest fate study in humans involved ingestion of sodium sulfite [Franz, F. and Sonntag, G. ARb. Kaiserl. Gesundheitsamt 28, 225 (1908)]. It was found that urinary sulfite first appeared 1/2 hour post-treatment, but only in negligible amounts (1%), an outcome attributed to rate-limiting degradation of organic-sulfite adducts. Moreover, intestinal resorption of sulfite was found to occur more rapidly than gastric resorption in rabbits [Rost, E. and Franz, F. Arb. Kaiserl. Gesundheitsamt 43, 187 (1912)]. In particular, a maximum lethal dose of sodium sulfite elicited increased gastric resorption, leading to typical sulfite intoxication in those animals unable to regurgitate, such as the rabbit. Lower levels of tissue intoxication in ruminants support this finding (70).

A 4 hour half-life for 3% sodium metabisulfite (LD_{50}) oxidation to inorganic sulfate was found for urine (19). A rate-limiting sulfite to sulfate oxidation would also explain the results of potassium metabisulfite feeding studies in rats. A 0.5% concentration increased urinary, but not fecal, calcium excretion. A 1% concentration increased fecal, but not urinary, calcium elimination. Since renal calcium excretion is difficult in rat, a gradient at the 0.5% metabisulfite level might depress higher anion concentrations such that excess calcium would be switched to colon elimination (66). There was no evidence of fecal excretion of labeled sulfur in dogs after inhalation tests (13). It is noteworthy that halving the splenic vein infusion of sodium bisulfite in dogs lowered serum bisulfite 2-3x(137). The half-value (80 mg/kg/hour) suggests a bisulfite to sulfate oxidation rate which is 4x the maximum conceivable exposure in humans with renal disease undergoing chronic intermittent peritoneal dialysis.

Oral Toxicity

The first monitored study of SO_2 intoxication reported in the literature found that sodium sulfite ingestion in humans (equivalent to ca. 18.5 mg/kg SO_2) induced acute gastric plethora. Lower doses (ca. 13.5 mg/kg SO_2) caused nonspecific gastric inflammation with vomiting, diarrhea, cyanosis, headache and vertigo. Still lower doses (ca. 3.5 mg/kg SO_2) elicited epigastric discomfort and frequent eructation [Rost, E. and Franz, F. Arb. Kaiserl. Gesundheitsamt 43, 187 (1912)].

No significant pathology was found in rats on diets containing sodium bisulfite at concentrations below 0.25% fed for 52 weeks. Between 0.25 and 2% levels there was evidence of stunting; clinical polyneuritis, "spectacle" eyes, uterine browning, bleached teeth, visceral atrophy, calcified renal tubular casts (but no tubular atrophy), testicular edema, bone and marrow atrophy and body weight loss. Secondary pathology included focal myocardial necrosis and fibrosis, mainly of the left heart, and gastric squamous hyperplasia (45). Lower bisulfite concentrations within the pathological range yielded less decrease in the survival time but the type of injury remained invariant and was unaffected by thiamine. There was no fatty liver degeneration. The lesions were more extensive after sulfate or sulfide diets. The higher metabolic inhibition by sulfide has been attributed to hexavalency (76).

There was evidence supporting the independent existence of metabisulfite ion in body fluids (21), but its pharmacology is invariant with bisulfite. Sodium metabisulfite equivalent to 350 or 750 ppm SO_2 fed to rats elicited no change in growth rate, behavior, general health, body weight, organ weight, adiposity, food intake, fertility, lactation or postnatal survival (84). Gastric mucosa were normal, with no evidence of the lesions reported in (45). Nor was there any change in ponderal or histological status of rats fed potassium metabisulfite (34). Reproductive rates through F_2 were unaffected. A 0.5-3% eosinopenia was reported. No tissue or serum lesions were found in normal and febrile infants, or infants with hepatopathies, fed sulfited jams (1000-3000 ppm SO_2) (102). Both mice and rats receiving 100 ppm SO_2 in grape juice as dietary supplement for 6 months showed weight loss, behavioral dysfunction, eosinopenia, adrenocortical hypertrophy, reduced glycogen and hepatocyte atrophy, subpleural hemorrhage, histiocytic infiltration, diarrhea,

gastric mucosal desquamation and distention and massive tissue plethora (33). The symptoms were invariant, but less severe, at a 50 ppm level.

A year of dietary sodium bisulfite elicited abnormal odontogenic epithelium and keratinization in a long term study on rats (67). Rats were given p.o. the potassium metabisulfite equivalent of a 70 kg human consuming an amount of fluid containing at least 1000 ppm SO_2 daily (80). Metabisulfite toxicity was found to vary directly with concentration, with an LD_{50} of 1800 mg/kg (equivalent to 1040 mg/kg SO_2). Cardiovascular collapse was preceded by a biphasic response in rats on metabisulfite diets: a generalized depression with prostration and anesthesia followed by episodes of gastric spasms, convulsions and tetanization (80). In contrast, potassium α-hydroxyethane sulfonate (the reaction product of acetaldehyde with potassium bisulfite) elicited an LD_{50} of 2700 mg/kg (equivalent to 1560 mg/kg SO_2). There was no evidence in this case that toxicity was a function of concentration. Nor was there evidence of specific lesion, but generalized plethora with foci of microscopic hemorrhage was conspicuous (80,111). Perhaps the earliest study of its kind offers counter evidence [Rost, E. and Franz, F. Arb. Kaiserl. Gesundheitsamt 21, 312 (1904)]. A glucose-sodium sulfite adduct was found to be twice as toxic as sodium sulfite itself, which led to the inference that SO_2 loses none of its toxicity in organic combination. A sodium bisulfite-benzoic acid diet in mice for 3 1/2 months with follow-up through F_4 provided support for mutual potentiation (125).

This was allegedly verified by wine studies in free and bound sulfurous acid with gastric juice assays, which indicted the bound acid (especially with glucose) as the toxic factor in hypochlorhydria (gastric HCl below 0.14%) (118,49). However, this result was denied by similar studies showing that the model juices in (118) were inadequately buffered and insensitive to relevant temperature and dilution effects (73). Given proper methodological adjustments, bound SO_2 was found to be highly stable, with minimal SO_2 liberated only in hyperchlorhydria due to the much higher SO_2 volatility in strongly acid solution. The requirement for differentiating bound and free salts has been rejected (63). Based on LD_{50} data in rats, there is a basic difference between the free and bound salt: toxicity of the free salt

(potassium metabisulfite) varied directly with concentration, while toxicity of the bound salt (bisulfite-acetaldehyde) did not (80). This result at least partially explains why the toxicity of the free agent would exceed that of the bound.

Coenzyme Interaction

SO_2 toxicity has been attributed to reduced sulfite oxidase activity elicited by thiamine deficiency (65,45). Up to 300 ppm daily in rats with sufficient vitamin B supplementation had no effect, while 50 ppm at the same rate in B-deficiency was eventually lethal. Up to 400mg SO_2 daily per avitaminotic person elicited no clinical alterations in GOT, GPT, LDH, α-HBDH, serum alkaline phosphatase or electrophoresis, thymol turbidity, hematocrit, erythrocyte count, motor conduction or reflex activity (62). Nor was there evidence of sulfite in blood or urine (98, 35).

Parenteral thiamine was not inactivated by SO_2, suggesting that anion-coenzyme interaction is confined to the gastrointestinal tract. SO_2 toxicity could be minimized by oral thiamine levels above the required, but a 5-fold or greater thiamine elevation above the normal did not raise the toxic SO_2 threshold (138,20). As is well known, sulfite addition to thiamine-free systems is toxic (5,122). It is also postulated that bisulfite degrades vitamin E, thus exposing vitamin A to degradation and elimination (67).

Parenteral Toxicity

Toxic tissue effects were first reported relative to protein coagulation, interference with blood supply and necrosis [Pfeiffer, L. Arch. Exp. Path. Pharmakol. 27, 261-96 (1890)]. Immediate respiratory depression, terminal clonic convulsions and death followed respiratory arrest 10-20 minutes post-injection i.v. sodium sulfite or bisulfite in mouse, hamster, rabbit and rat (65). Evidence of SO_2 depression of CNS centers was given in (111). An MLD of 106 mg/kg sodium bisulfite i.v. in rabbits induced an instantaneous CNS effect, with clonic tremors, rapid hypotension and death in a few minutes. The histopathology was negative (114). Low doses of ambient SO_2 had no effect on conditioned reflex activity, but higher

doses increased the latent period 2-2 1/2 x (134). Restlessness and irritability were also symptomatic at various concentrations (91,6) but other data was negative (90). Growth and survival rates of guinea pigs exposed to 5.7 ppm SO_2 in ambient air for 1 year was significantly higher for both sexes. At 0.13 ppm the females had a growth rate below controls after 52 weeks, and the males only after 14 weeks, exposure (4). There was no effect on rat estrus (89).

Guinea pig lung after long-term (1 year) SO_2 exposure (0.13-5.7 ppm) showed little evidence of spontaneous lesion, using the absence of histiocytic infiltration in alveolar walls as index. There was focal nodular lymphoid hyperplasia around the airways at 52 weeks. The degree of tracheitis varied directly with dose at 0.13-1.01 ppm levels, with submucosal neutrophil and histiocyte invasion, but not after 5.7 ppm (4). Nephritis and nephrosis, primarily in males, was more frequent after inhalation exposure to 0.13-1.01 ppm for 1 year than after 5.72 ppm. Hepatocyte vacuolization varied directly with dose after a year-long exposure. Absence of inflammation or emphysema and minor hemorrhage, edema and epithelial infiltration was also reported (91,92) for rats and rabbits and guinea pigs (6).

Tidal volume, respiratory rate, minute volume, dynamic compliance, pulmonary flow resistance and the work of breathing gave values which fluctuated widely and were completely independent of low and long-term (4) or high and short-term (82) exposures of guinea pigs to SO_2 in ambient air. Even at 180 ppm recovery of tidal volume to the control levels required only 30-60 minutes, or less than the exposure interval, suggesting a type of reflex adaptation. Apparent behavioral adaptation was found also after 8 hours exposure to 89 ppm (6).

Corneal exposure to SO_2 aerosols and sodium bisulfite perfusions in vital rabbit eye systems (55) led to epithelial insult resembling acid burns and stromal-endothelial injury resembling alkali burns (56). It is significant that a marked eosinophilia in exposed corneal infiltrates differentiates SO_2 inflammation from typical acid (55).

Increased mitosis and tracheobronchial goblet cell number and size, indicative of sialomucin production, appeared up to 5 weeks post-treatment in rats exposed to 400 ppm SO_2 in air for a 90 hour maximum over 6 weeks. Although the trauma resembled chronic bronchitis, there was no evidence of histological damage. The tracheobronchial secretion was thought to represent a response to surface epithelial irritation via a vagus-mediated reflex (79).

In human aerosol studies chronic exposure to 25 ppm SO_2 from 1-19 years did not effect blood pressure, ponderal status, radiology or vital capacity; in fact, hilar and extrahilar calcification and vital capacity were even improved over 135 unexposed subjects working in an oil refinery environment (8). Tolerance in humans was reported after long-term exposure to ambient SO_2 (115) but not in a patient with SO_2-induced urticaria (110). Inhalation effects in humans included a lymphocytosis, simulating low grade chronic infection, and elevated urinary acidity. There was no other significant pulmonary histopathology and no relation between symptomatology and exposure. It was concluded that SO_2 intoxication is primarily an acid storage disease, marked by fatigue, shortness of breath and acidic urine (72).

Hematology and Enzymology

Sodium metabisulfite (10^{-3}M) was 60% inhibitory of serotonin degradation by ceruloplasmin, an effect congruent with ascorbic acid, glutathione and other reducing agents (143). Serum ceruloplasmin and erythrocyte sedimentation rate were higher in combined sodium bisulfite-benzoic acid diets; but complement, phagocytosis and blood ketone level were lower (125). Blood alkalinity, C-reactive protein and hematology were normal. Bovine prothrombin and autoprothrombin C were inhibited by both sodium sulfite and bisulfite after a 2 hour treatment, while thrombin extinction required 24 hours (28). In rabbit studies, 5 ppm SO_2 in air for 20 minutes daily for 5 weeks elicited an initial increase in body weight, blood count, Hb and hematocrit with rapid recovery. Doses 10 ppm or higher decreased the value of these variables with slow recovery, while 3 ppm was without effect (100).

Hematocrit, Hb, erythrocyte and leukocyte count, SGOT, SGPT and blood urea nitrogen remained unchanged in guinea pigs after a 1 year exposure to 0.13-5.7 ppm in air (4). A linear Hb increase with SO_2 concentrations between 6 and 20 ppm, but non-linear above 20 ppm, was found for guinea pigs in inhalation studies (82).

Inhibition of glutamic dehydrogenase, α-glycerophosphate dehydrogenase, liver alcohol dehydrogenase, malic dehydrogenase and myocardial lactic dehydrogenase occurred at "sulfite" concentrations of 2-50 gamma (109). Decreases were reported for histidine deaminase (126), catalase (55), lactic dehydrogenase (78), lactic dehydrogenase isozymes (21), inorganic serum phosphate (77), liver aminotransferases (78), bovine erythrocyte acetylcholinesterase (105), pyridoxal phosphate (1), adrenal ascorbic acid (90), serum oxidase (33), oxidized and reduced glutathione (11), alkaline phosphatase (33), liver glycogen (128,33) and peroxidase (93). Increases were reported for adrenal ascorbic acid (33), reduced glutathione (12), urinary 17-ketosteroids and pituitary-adrenal hyperfunction (90), liver, brain and serum aldolase (78), serum alkaline phosphatase (77) and hyperglycemia (128,76,43). Increased erythrocyte glucose and lactate production due to bisulfite activation of phosphofructokinase was reported [Duhm, J., Deuticke, B. and Gerlach, E. Hoppe-Seyler's Z. Physiol. Chem. 350(8):1008-16 (1969)]. Decreased erythrocyte oxygen consumption was also found (11). The protective properties of SO_2 for labile amino acid hydrolysis (especially tryptophan) have been noted (97). Eosinophilia was reported at statistically significant levels (90,55). No change was found for glutathione (90), glutamic aspartic or glutamic alanine transaminase at neutral pH (1), serum calcium (77), or phagocytic index (90).

Hyperglycemia and bilirubinemia were indices of moderate toxicity of parenteral sodium metabisulfite in the dog, cat, mouse, rat and rabbit after acute dosing. Rapid host inactivation invariably followed. In chronic administration (daily i.p. or i.v. for 2 weeks) there was anemia and moderate liver dystrophy and vacuolization (43). The toxicity was thought to result from contact irritation of smooth mucosa in the gastrointestinal and respiratory tracts and the resorptive activity after coupling with aldehyde and ketone groups in tissue. Sodium hydroxymethane sulfonate was found to inhibit glycolate oxidation by glycolic oxidase (144). The sulfonate's

low dissociation constant and the competitive kinetics of glycolate decay would suggest that sodium bisulfite itself is not the responsible inhibitor. The sulfonate reduction of glyoxalate to glycolate by muscle lactic dehydrogenase was without effect, but bisulfite was competitively inhibitory. Bisulfite alone also effectively inhibited pyruvate reduction, lactate oxidation, and muscle lactic dehydrogenase.

A slight erythrocytosis, moderate leukopenia, slight eosinopenia and slight lymphocytosis followed acute dosing with ambient SO_2 exposure in guinea pigs. A slight erythrocytopenia, leukopenia and neutropenia, but modest lymphocytosis and monocytosis was harvested by chronic dosing in the same system. Combined administration with ammonia suppressed SO_2 toxicity (78), which tends to confirm the "acid-storage" outcome in sulfited tissues.

Serology

Controlled inhalation of SO_2 by rabbits for 113 days with concurrent i.v. pneumococcal immunization for a 48-day interval elicited a sharp week-long elevation in complement beginning with the second week post-treatment. Titer decrease and stereotypy was almost equally sharp through the remaining 3 1/2 weeks of immunization. The ascending curve for the control animals (not receiving SO_2) mirrored the test result, but onset was a week later; significant titer decrease did not begin until after immunization and was gradual. Agglutinins were also significantly inhibited by SO_2. A booster dose of the same antigen 100 days following the end of immunization elicited little or no response in the test group, while titer ascent for the control groups was nearly as high as the original exposure (9). Similar results were obtained for α-globulins in inhalation studies, where the highest hemagglutination titer confirmed that the antibody level was extremely low (91). However, in experiments showing SO_2 inhibition of the bactericidal properties of whole rabbit blood, complement fixation was relatively unchanged (74). *In vitro* polymorphonuclear leukocyte chemotaxis was inhibited by M/8 sodium bisulfite, although the two necessary conditions for migration - complement and calcium ions - remained unchanged. Bisulfite was thought to

act directly on neutrophil enzyme assembly, whose peroxidase is known to be increased in amount and activity during cell mobility.

Salt Effect and Cell Response

Studies of SO_2 gas on mouse fibroblast, mouse liver parenchymal cell and HeLa cultures were paradoxical. Fibroblast and parenchymal cells were insensitive to 500 ppm but HeLa proliferation was detected. Significant fibroblast and parenchymal loss followed 1000 ppm, while growth of all cell types was 75-90% inhibited by 2000 ppm. The parenchymal culture was least sensitive at the highest SO_2 concentration. These results were functions of a "biological" system with 40% serum. An equivalent effect was achieved with 10x lower SO_2 concentrations and only 10% serum in semi-synthetic medium (130). Serum must contribute an important prophylactic effect. If there is a combination between some organic serum component and anion, it would suggest that the SO_2 effect is not mediated via direct action of increased salt concentration on cells. There is evidence that SO_2 is fixed chiefly by cells and that, in the plasma, fixation as sulfite occurs without plasma protein involvement (23). Both reduced growth and increased mortality rate were also found to be dose-and time-dependent with sodium salts of SO_2, suggesting that the toxicity is mediated by some other pathway than by combination to produce the various salts. Growth rate change after salt exposure was in general not due to pH alteration, except for the highest dose of sodium bisulfite (200mg%), during which the cultures were shifted to non-physiological ranges. Both sodium bisulfite and sodium sulfite potentiated cell growth in the middle doses (50mg%), while inhibiting at lowest (10mg%) and highest (200mg%) exposures (130). No tolerance was found except at low exposures (5 ppm) with appropriate recovery periods. HeLa sensitivity was higher than that for the other cell systems, especially reflecting that bisulfite is more toxic than sulfite and that human tissue is more sensitive than that of lower animals. Retardation of small circulating lymphocyte mitosis in cultures from humans after SO_2 inhalation exposure led to blastoid enlargement after an initial reduction of mean cell diameter (119). SO_2 intoxication was most effective during the interval of DNA start-up.

Mutagenicity

Bisulfite ion was found to convert 5-methylcytosine to thymine at pH 6 and 37° and to induce mutation at *c* genes of lambda phage at pH 5.6 (59,123). The mutation frequency was a function of time up to 90 minutes, after which longer treatment was without effect. Adenine and guanine were unreactive. The sulfited cytosine adduct can be deaminated irreversibly to 5,6-dihydrouracil-6-sulfonate, which is desulfited at strongly alkaline pH to yield uracil. Decreased DNA synthesis and mitotic index and deficient karyotype have also been reported (119). It has been suggested, but otherwise unexplored, that normal tissue could mutate to neoplastic states via "atrophic" anerobiosis under redox conditions of an autonomous thiorespiratory metabolism (140). Retardation of chick embryo blastoderm and complete absence of embryogenesis occurred after a 2 hour exposure to SO_2 (7).

For a comprehensive report on recent experimental developments in reproductive effects of sulfiting agents, see *Teratogenic Evaluation of Sodium Metabisulfite and Sodium Bisulfite in Mice, Rats, and Hamsters*, Food and Drug Research Laboratories, Waverly, New York.

Carcinogenicity

Sodium bisulfite has been found to potentiate carcinomatous foci. Dietary bisulfite (0.4%) doubled Ehrlich ascites cell growth in mice and halved the survival time (41). Administration of 80 mg/kg sodium bisulfite per day 3 months after i.p. implantation of Ehrlich ascites tumor in mice led to a tumor proliferation higher than that in animals receiving either bisulfite-benzoic acid combination or benzoic acid alone (125).

Aeration studies have confirmed that protracted exposure to SO_2 at relatively high concentrations (500 ppm) led to larger and more frequent (2x) primary pulmonary tumors in mice at earlier ages than untreated controls (106). Since only the larger masses become malignant, SO_2 is assigned precursor responsibility: alveolar hyperplasia with lymphatic engorgement. If the hyperplasia represents an initial inflammatory reaction and invasive leukocytosis a subsequent tolerance phenomenon (115) (which free radicals do not elicit), then the tolerance interval must be associated with conditions favorable to tumor potentiation.

Conclusion

The dissociation pattern of "sulfiting agents" follows the successively increasing pH environments in descending through the digestive tract, from SO_2-hydrate to bisulfite to sulfite, at which point catalytic oxidation occurs. Approximately half of each dose of sulfiting agent is so oxidized by the small intestine before resorption, with rapid renal clearance. Some fraction of the remainder - probably very large, since evidence of biliary sulfites is negative - is oxidized by the liver after resorption, also with facile renal elimination. But some unknown fraction escapes both duodenal and hepatic detoxification to become serologically significant. There is evidence for both disulfide bond sulfitolysis with formation of S-sulfonates (and subsequent maintenance of low plasma sulfite levels in controlled steady-state reactions) and reactivity with aldehyde and ketone groups to yield sulfite adducts in plasma.

Although sulfonate inhibition of enzyme systems, principally dehydrogenases affecting carbohydrate metabolism, has been established, sulfite immobilization is prophylactic against subsequent tissue invasion. Accordingly, SO_2-or its salts-has a nonspecific effect on host tissue, whose only common histopathology after exposure is plethora with exudate formation (even among siblings stigmatized with congenital sulfite oxidase deficit). Low phagocytic indices confirm the absence of significant fixed cell lesion. So also does the peripheral blood picture after both acute and initial chronic exposure to both high and low concentrations: erythrocytosis. This and neutropenia with relative lymphocytosis. This hematology typifies acidemia or acidosis. Subsequent chronic administration of either high or low concentrations leads to erythropenia with relative leukocytosis. Together with evidence of no pCO_2 elevation and inhibition of both complement fixation and agglutinin titer, this picture suggests an alkalosis. It would follow that the primary effect of undetoxified SO_2 or SO_2 salts is a disturbance of acid-base equilibria.

In general, there is some lowest dose for each experimental system which is either ineffective or mildly inhibitory, an intermediate dose which is metabolically stimulatory and some highest dose which is metabolically inhibitory. The lowest concentrations are invariably dose-dependent, while response to higher doses is not. If a biochemical variable continues

to increase or decrease linearly on continued dosing, there is some level above which the response becomes non-linear. If two biochemical variables vary inversely up to a certain anion concentration, there is some threshold above which both will continue to vary inversely, but each in a direction opposite to its initial one. This reversal effect, found especially in the imcompatible reports of eosinophil harvest, is probably due to enzyme switching elicited by altered acid-base balance. The pattern is compatible even for those systems using concentrations within the FAO/WHO range of unconditional acceptable daily intake.

CHEMICAL INFORMATION

SULFUR DIOXIDE

I. Nomenclature

A. Common names: sulfur dioxide; sulfurous anhydride; sulfurous acid ahnydride; sulfurous oxide; sulfur oxide (ambiguous)

B. Chemical name: sulfur dioxide

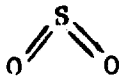
C. Trade name: none (the process of treating foods with sulfur dioxide or its derivative salts is often referred to, especially in the older literature, as "sulfiting," "sulfuring," "sulfurizing," or "sulfurating")

D. CAS Reg. No. 7446095

II.. Empirical Formula

SO_2

III. Structural Formula



IV. Molecular Weight

64.07

V. Specifications

A. Food Chemicals Codex: not listed

B. USP

SO_2- 97% by volume min

non-volatile residue- 25 ppm max

H_2O- 2% max

C. Food Grade (as reported by one firm)

SO_2- 99.98% min

H_2O- 100 ppm max

acidity (as H_2SO_4)- 10 ppm max

non-volatile residue- 50 ppm max

misc.- the product should be free of all other gases and foreign matter

VI. Description

A. General: colorless gas or liquid with a characteristic, pungent, suffocating odor

B. Physical properties

m.p. -76.1°C

b.p. -10°C

vapor pressure= 3.2 atm at 68°F

solubility

H_2O- 11.3 g/100g at 20°C

22.8 g/100g at 0°C

ethanol- 96 vol/vol at 20°C

acetone, chloroform, ether, formic acid, acetic acid- soluble

C. Stability

1. susceptible to both oxidation (by air, in solution, pH dependent) and reduction (by microorganisms)
2. reacts (as sulfite or bisulfite), often reversibly, with many food components, such as aldehydes and sugars; irreversibly, with thiamine (Vitamin B_1)
3. the aqueous solution, being moderately acidic, corrodes active metals such as some types of stainless steel

SODIUM BISULFITE

I. Nomenclature

A. Common Names: sodium bisulfite; sodium hydrogen sulfite; sodium acid sulfite

B. Chemical names: sulfurous acid, monosodium salt; sodium bisulfite; sodium hydrogen sulfite

C. Trade name: none

D. CAS Reg. No. 7631905

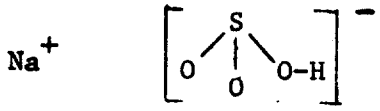
II. Empirical Formula

$NaHSO_3$

Note: The existence of sodium and/or potassium bisulfite as discreet compounds seems highly doubtful. It has been stated that "sodium hydrogen sulfite, $NaHSO_3$, does not exist as a solid" (Inorg. Synth., Vol. II, W. C. Fernelius, ed. McGraw Hill, 1946, p. 164), while, in aqueous solution, pyrosulfite is hydrated to bisulfite and is not present in dilute solutions. Most of the sodium bisulfite sold commercially is actually the anhydride, sodium metabisulfite, which is less hygroscopic and thus more stable to

storage and transport (Kirk-Othmer Encyc. Chem Technol. 2nd ed., Wiley, New York, 1929).

III. Structural Formula



IV. Molecular Weight

104.06

V. Specifications

A. Food Chemicals Codex

assay- not less than 58.5 nor more than 67.4% of SO_2

limits of impurities-

arsenic (as As)-3 ppm

heavy metals (as Pb)- 10 ppm

iron- 50 ppm

selenium- 30 ppm

B. Chemical Grade

1. Fisher Chemical Co. (ACS reagent)- a mixture of $NaHSO_3$ and $Na_2S_2O_5$

assay- 58.5% min of SO_2

limits of impurties

insolubles- 50 ppm

arsenic- 1 ppm

chloride- 500 ppm

heavy metals (as Pb)- 10 ppm

iron- 20 ppm

2. J. T. Baker Chemical Co. (ACS reagent)

same as above except chlorides- 200 ppm

VI. Description

A. General: (from the Food Chemicals Codex) "sodium bisulfite consists of sodium bisulfite ($NaHSO_3$) and sodium metabisulfite ($Na_2S_2O_5$) in varying proportions. It occurs as white or yellowish-white crystals or granular powder having an odor of sulfur dioxide."

B. Physical properties

m.p.- dec.

solubility

H_2O- 1 g/4 ml (cold)

ethanol- sl. s.

C. Stability
unstable in air (oxidizes)

SODIUM METABISULFITE

I. Nomenclature

A. Common names: sodium metabisulfite; sodium pyrosulfite

B. Chemical names: pyrosulfurous acid, disodium salt; sodium metabisulfite; sodium pyrosulfite

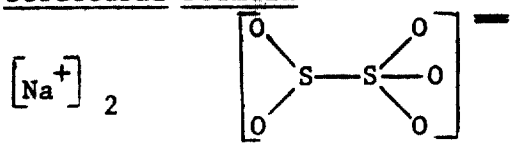
C. Trade name: none

D. CAS Reg. No. 7757746

II. Empirical Formula

$Na_2S_2O_5$

III. Structural Formula



IV. Molecular Weight

190.10

V. Specifications

A. Food Chemicals Codex

assay- 90% min

limits of impurities

arsenic (as As)-3 ppm

heavy metals (as Pb)- 20 ppm

iron- 20 ppm

selenium- 30 ppm

B. Chemical grade

1. Fisher Chemical Co. (ACS reagent)

assay- 97% min

limits of impurities

insolubles- 50 ppm

arsenic- 1 ppm

chloride- 500 ppm

heavy metals (as Pb)- 10 ppm

iron- 20 ppm

thiosulfate- 500 ppm

2. J. T. Baker Chemical Co.

   same as above

3. MC&B Manufacturing Chemists

   same as above except heavy metals- 20 ppm and no thiosulfate spec

VI. Description

A. General: colorless crystals or a white to yellowish crystalline powder with the odor of SO_2

B. Physical properties

   m.p.- ?

   solubility

   H_2O- 6 moles/100 moles H_2O at 20°C

   ethanol- sl. s.

C. Stability

   easily oxidized in air when not thoroughly dry

SODIUM SULFITE

I. Nomenclature

A. Common names: sodium sulfite; anhydrous sodium sulfite; exsiccated sodium sulfite

B. Chemical names: sulfurous acid, disodium salt; sodium sulfite; sodium sulfite, anhydrous

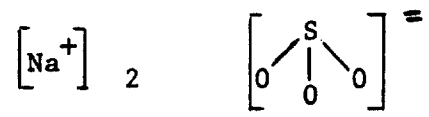
C. Trade name: none

D. CAS Reg. No. 7757837

II. Empirical Formula

Na_2SO_3

III. Structural Formula



IV. Molecular Weight

126.04

V. Specifications

A. Food Chemicals Codex

assay-95% min

limits of impurities

arsenic(as As)- 3 ppm

heavy metals(as Pb)- 10 ppm

selenium- 30 ppm

B. Chemical grade

1. Fisher Chemical Co.(ACS reagent)

assay- 98% min

limits of impurities

insolubles- 50 ppm

free alkali(as Na_2CO_3)- 1500 ppm

chloride- 200

arsenic- 0.5

heavy metals(as Pb)- 10 ppm

iron- 10 ppm

2. MC&B Manufacturing Chemists (ACS reagent)

assay- 98 % min

limits of impurities

arsenic- 1 ppm

chlorides- 200 ppm

heavy metals (as Pb)- 10 ppm
iron- 10 ppm
insolubles- 50 ppm

3. J. T. Baker Chemical Co. (ACS reagent)
assay- 98% min
limits of impurities
insolubles- 50 ppm
free alkali (as Na_2CO_3)-1500 ppm
chlorides- 100 ppm
arsenic- 0.5 ppm
heavy metals (as Pb)- 10 ppm
iron- 5 ppm

VI. Description

A. General: white or tan to sl. pink, odorless or nearly odorless powder

B. Physical properties
m.p.- dec. red heat
solubility
H_2O- 1 g/4 ml (cold)
ethanol- sl. s.
$d^{15.4} = 2.633$

C. Stability
oxidizes in air and hydrates below 35°C

POTASSIUM BISULFITE

I. Nomenclature

A. Common names: potassium bisulfite; potassium hydrogen sulfite; potassium acid sulfite

B. Chemical names: sulfurous acid, monopotassium salt: potassium bisulfite; potassium hydrogen sulfite

C. Trade names: none

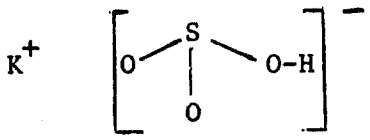
D. CAS Reg. No. 7773037

II. Empirical Formula

$KHSO_3$

Note: see note for sodium bisulfite

III. Structural Formula



IV. Molecular Weight

120.17

V. Specifications

A. Food Chemicals Codex: not listed

B. Chemical grade: not listed

VI. Description

A. General: colorless crystals

B. Physical Properties

m.p. 190°C (dec.)

C. Stability

?

POTASSIUM METABISULFITE

I. Nomenclature

A. Common names: potassium metabisulfite; potassium pyrosulfite

B. Chemical names: pyrosulfurous acid, dipotassium salt; potassium metabisulfite; potassium pyrosulfite

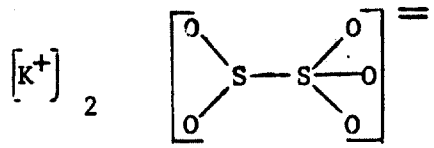
C. Trade name: none

D. CAS Reg. No. 4429429

II. Empirical Formula

$K_2S_2O_5$

III. Structural Formula



IV. Molecular Weight

222.23

V. Specifications

A. Food Chemicals Codex

assay- 90.0 % min

limits of impurities-

arsenic (as As)- 3 ppm
heavy metals (as Pb)- 10 ppm
iron- 10 ppm
selenium- 30 ppm

B. Chemical grade

1. Fisher Chemical Co. (reagent grade)
insolubles- 100 ppm max
chloride- 100 ppm
arsenic- 5 ppm max
heavy metals (Pb)- 30 ppm max
iron- 10 ppm max

2. MC&B Manufacturing Chemists
assay- 95% min
limits of impurities
arsenic- 1 ppm
chloride- 50 ppm
heavy metals (as Pb)- 30 ppm
insolubles- 50 ppm
iron- 10 ppm

3. J. T. Baker Chemical Co.
same as above except heavy metals- 10 ppm

VI. Description

A. General: white or colorless crystals, crystalline powder or granules with SO_2 odor.

B. Physical properties
m.p.- 190°C (dec.)
solubility
H_2O- 3.5 moles/100 moles H_2O at 20°C
ethanol- sl. s.
d. = 2.34

C. Stability
oxidizes in air

POTASSIUM SULFITE

I. Nomenclature

A. Common name: potassium sulfite

B. Chemical names: sulfurous acid, dipotassium salt; potassium sulfite

C. Trade name: none

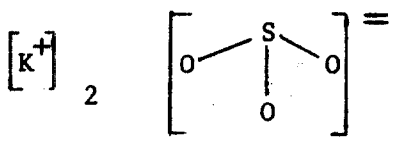
D. CAS Reg. No. 10117381

II. Empirical Formula

K_2SO_3

Note: it is listed as a dihydrate in the Merck Index and the Handbook of Chemistry and Physics, but as the anhydrous salt in the Food Chemicals Codex. It has been reported not to form a hydrate (Inorg. Synth. Vol. II, W. C. Fernelius, ed., McGraw-Hill, 1946, p. 167).

III. Structural Formula



IV. Molecular Weight

158.27

V. Specifications

A. Food Chemicals Codex

assay- 90.0% min

alkalinity (as K_2CO_3)- 0.25-0.45%

limits of impurities

arsenic (as As)- 3 ppm

heavy metals (as Pb)- 10 ppm

selenium- 30 ppm

B. Chemical grade

1. Fisher Chemical Co.

chloride- 1000 ppm max

arsenic- 1 ppm max

iron- 10 ppm max

heavy metals (Pb)- 10 ppm max

2. MC&B Manufacturing Chemists (reagent grade)

arsenic- 3 ppm max

heavy metals (Pb)- 10 ppm max

selenium- 30 ppm max

VI. Description

A. General: white, odorless, granular powder

B. Physical properties

m.p.- ?

solubility

H_2O- 12 moles/100 moles H_2O

ethanol- sl. s

C. Stability

oxidizes in air

VII. Analytical Methods

All of the analytical methods noted below are concerned with the estimation of available SO_2 (free and/or bound) regardless of the actual "sulfiting agent" added.

A. Colorimetric methods

1. direct (no separation of SO_2 from the food milieu is necessary)

(a) AOAC, 1970, p. 349

dried fruit- the sample is blended with water, treated with base (to liberate bound SO_2), acidified, complexed with sodium tetrachloromercurate and determined colorimetrically by the fuchsin-formaldehyde reaction. The method is an AOAC official final action.

(b) AOAC, 1970, p. 163

beer- the sample is stabilized with mercuric chloride and determined colorimetrically by the fuchsin-formaldehyde reagent; accuracy, ± 5% at 1-75 ppm; AOAC official action.

(c) Ref. 24

beer- the SO_2 is reduced with $SnCl_2$ to H_2S which is converted to methylene blue with p-aminodimethylaniline and determined colorimetrically; determines 1-20 ppm in a 5 ml sample with 2-significant-figure accuracy

(d) Ref. 29

white sugar-total SO_2 is determined by the fuchsin-formaldehyde reaction; accurate and reproducible below 1 ppm to more than 30 ppm

(e) Ref. 47

wines, fruit juices, pectin, dried fruits and sirups - SO_2 is liberated, complexed with mercuric chloride and determined colorimetrically with fuchsin-formaldehyde; stand. dev., 1-6%

(f) Ref. 48

biscuit flour (not good for bread or cake flour)- SO_2 is determined colorimetrically by the decolorization of Astrazone Pink FG(Bayer); the sensitivity threshold is 30 ppm

(g) Ref. 52

white sugar- fuchsin-formaldehyde colorimetry; determines 0-10+ ppm on a 40 g sample

(h) Ref. 54

tissue extracts, including blood- an alkaline tissue extract (blood requires mercuric chloride treatment) is determined colorimetrically with fuchsin-formaldehyde; gives good recovery of added SO_2 in the 150 ppm range

(i) Ref. 86

wines, red and white- SO_2 is liberated with alkali, stabilized with sodium tetrachloromercurate and determined colorimetrically by fuchsin-formaldehyde; range investigated, 20 -300 ppm; agrees well with iodometric titration

(j) Ref. 129

beer- free SO_2 is determined by direct reaction with fuchsin -formaldehyde; total (and thus bound, by difference) SO_2 is determined by acid treatment (sodium tetrachloromecurate stabilization), base treatment and then fuchsin-formaldehyde colorimetry; accurate at the 1-15 ppm level

(k) AOAC, 1970, p. 349

meat- treat the ground sample with malachite green reagent; decolorization indicates sulfitation of the meat; AOAC official final action

2. indirect (requires separation of SO_2 from the food milieu)

(a) Ref. 15

malt, beer- SO_2 is distilled from the sample in N_2, trapped by sodium tetrachloromercurate and determined by the fuchsin-formaldehyde procedure using a precision spectrophotometer (such as Beckmann DU); accurate to ± 5% at 1-75 ppm

(b) Ref. 53

brandy- SO_2 is distilled from the sample in CO_2 or N_2 and then determined by the Brenner method- A.1.(c) Ref. 24

(c) Ref. 58

hop cones- distill SO_2 from phosphoric acid solution and determine by fuchsin-formaldehyde colorimetry; sensitive at the 10 ppm level

(d) Ref. 68

various foods- SO_2 is separated by microdiffusion (from phosphoric acid into sodium tetrachloromercurate-glycerine; requires about 90 min) and then determined by fuchsin-formaldehyde colorimetry; stand. dev. under 5% for less than 12 ppm

(e) Ref. 83

fruit juices and soft drinks- free SO_2 is desorbed by N_2 entrainment at pH 1-2 and determined by fuchsin-formaldehyde colorimetry; suitable for less than 50 ppm

(f) Ref. 99

fruits and fruit products (sirups and beverages)- total SO_2 is liberate with alkali, distilled from acid solution and determined colorimetrically by decolorization of the peroxodisulfatotitanic acid reagent (yellow to colorless); interferences are negligible and recoveries vary from 90-98% at the 50-400 ppm level

(g) Ref. 135

soft drinks, carbonated and non-carbonated- the Technicon Auto-analyzer is used to distill the sample, absorb the SO_2 in sodium tetrachloromercurate, and determine it colorimetrically by the fuchsin-formaldehyde reaction; it is described as a reproducible, rapid screening method for soft drink quality control; total SO_2 is determined, but a modification involving dialysis rather than distillation to determine free SO_2 is discussed

B. Titrimetric methods

1. direct

(a) Ref. 25

wine- free SO_2 is titrated amperometrically with I_2 in the cold in acid medium; total SO_2 may also be determined; the method is accurate to within a few ppm at the 20-300 ppm level

(b) Ref. 32

preserved fruit- a basic suspension of the preserved fruit is directly titrated iodometrically; any substance reducing iodine under the given conditions would interfere; the method is intended for a rapid approximation at the 500-3000 ppm range and is suitable for an in-the-field analysis

(c) Ref. 107

fresh pork sausage- the sample is macerated with water, treated with alkali and divided into two portions; the first is acidified and titrated iodometrically; the second is acidified, treated with peroxide and then titrated, thus providing an interference blank; the coeff. of variation is 8%, the results agree closely with standard

methods and the method is proposed for rapid quality control procedures

2. indirect

(a) AOAC, 1970 pp. 348, 349, 191, 398

wine, meat extracts and other food materials (excepting dried onions, leeks and cabbage)- the modified Monier-Williams method in which the acidified sample is distilled with N_2 under reflux into hydrogen peroxide and the formed sulfuric acid titrated with sodium hydroxide and/or determined gravimetrically as barium sulfate is the official AOAC method; other volatile sulfur compounds do not interfere

(b) Ref. 10

beer- a modified Monier-Williams method in which the acidified sample is distilled with CO_2, the liberated SO_2 is absorbed in H_2O_2 and titrated with 0.02 N NaOH (1 ml= 12.8 ppm SO_2); the method is recommended for adoption by the Institute of Brewing Analysis (England)

(c) Ref. 27

cider- total SO_2 is distilled from the acidified sample in N_2, absorbed in standard iodine and titrated with thiosulfate; volatile I_2 reducing substances could interfere but were not found in the samples tested; reproducibility was ± 0.6 ppm in the 100+ ppm range

(d) Ref. 26

cider- free SO_2 is desorbed from the acidified sample at room temp. by air, trapped and oxidized in H_2O_2 and titrated with alkali; volatile acids, such as acetic, in the cider will intefere-estimated to be less than 2.5 ppm in normal cider; they claim complete recovery of free SO_2 with better than 0.5 ppm reproducibility at the 10-50 ppm level; blank values due to dissociation of bound bisulfite (mainly with pyruvate) usually range from 1-10 ppm

(e) Ref. 37 & 38

wines and grape juice- free and combined SO_2 are separated by distillation of the sample at different temperatures and then titrated iodometrically; accuracy, ca. 3.8 for free SO_2 at 10 ppm and 0.3% for combined at 100-300 ppm

(f) Ref. 40

wine- total SO_2 is distilled from the sample and determined iodometrically; the usable range is from 12-235 ppm with a precision of ± 2 ppm

(g) Ref. 46

gelatin- total SO_2 is steam distilled from the acidified sample into H_2O_2 and determined by alkalimetry; accurate to ± 8 ppm

(h) Ref. 83

fruit juices and soft drinks- free SO_2 is desorbed from the sample with N_2 at pH 1-2, trapped in alkaline glycerol and determined iodometrically; applicable to samples with greater than 50 ppm

(i) Ref. 87

dried apricots and peaches- free SO_2 is desorbed under partial vacuum from the acidified sample, trapped in H_2O_2 and determined alkalimetrically

(j) Ref. 104

beer, wine, distilled liquors- the acidified sample is placed in a Conway microdiffusion cell and the diffused volatile acids are titrated with $Ba(OH)_2$ to give total volatile acidity; then the process is repeated but with the addition of HgO to complex SO_2 and thus provide a blank; with beer the recovery of added SO_2 varied from 90-170%; the author recommends the analysis for three reasons: small sample required, short analysis time and agreement of results with the AOAC method

(k) Ref. 112

white wine- air is drawn through the acidified sample at 20°C or below, passed through a reflux condenser and the free SO_2 is trapped in peroxide and titrated with NaOH; combined SO_2 can then be determined on the same sample by boiling the solution; acetic acid, if present at more than 0.12% levels, will interfere; the method is described as more accurate than iodometry and is widely used in the Australian wine industry; not applicable to red wine

(l) Ref. 113

wine- a distillation-iodometry procedure

(m) Ref. 136

biological fluids (serum, saline-) - SO_2 is separate from the milieu by Conway-type microdiffusion, oxidized with peroxide and determined alkalimetrically; poor, but reproducible recovery (76-82%) is achieved from serum at the 100-500 ppm level

C. Other methods

1. electrical

(a) Ref. 40

fruit juices and wines- no separation step is required but a preliminary qual. gas chromatographic analysis is needed; determination is via an "osmopile," a special galvanic battery

(b) Ref. 40

wine- no separation step is required; SO_2 is determined polarimetrically at the 1-50 ppm level with a precision of $\pm$0.7 to 11.5 ppm; no interferences were reported

2. wet methods

(a) Ref. 94

biological fluids- SO_2 is reacted with radioactively labeled N-ethylmaleimide; the solution is paper chromatographed, the proper spot located and counted by liquid scintillation (PPO and POPOP fluors); the method is reported to be as sensitive as the fuchsin-formaldehyde reaction; and 0.1 ml sample containing 5 mμ moles (4 ppm) is required for accurate results

(b) Ref. 145

foods- an indicator paper based on the starch-I_2 complex is used to determine from 5-1000 ppm SO_2 by color comparison with standards

D. Miscellaneous

Ref. 36

The author, who has devoted considerable effort to the study of SO_2 determination in wines, states that there are inherent inaccuracies in the differentiation between free and bound SO_2 by distillation procedures. This is a consequence of slow dissociation of bound SO_2, especially in colored wines.

VIII. Occurrence

A. Plants- none

B. Animals- sulfite is present in bovine seminal plasma at a level of about 80-100 ppm(81).

C. Synthetics-none

D. Natural inorganic sources- SO_2 is naturally present in volcanic gases and is, of course, a major component of air pollution

BIOLOGICAL DATA

I. Acute toxicity

1. Ref. 137 (sodium bisulfite)

	Mouse		*Rat*						*Rabbit*		*Dog*	
	1.25 Gm. $NaHSO_3$ per 100 ml. solution		*25 Gm. $NaHSO_3$ per 100 ml. solution*		*5 Gm. $NaHSO_3$ per 100 ml. solution*		*1.25 Gm. $NaHSO_3$ per 100 ml. solution*		*25 Gm. $NaHSO_3$ per 100 ml. solution*		*25 Gm. $NaHSO_3$ per 100 ml. solution*	
	Dose (mg./Kg.)	*Deaths*	*Dose (mg./Kg.)*	*Deaths*	*Dose (mg./Kg.)*	*Deaths*	*Dose (mg./Kg.)*	*Deaths*	*Dose (mg./Kg.)*	*Deaths*	*Dose (mg./Kg.)*	*Deaths*
	300	0/4	300	0/4	500	0/4	500	0/4	133	0/4	174	0/4
	450	0/4	450	1/4	654	4/4	654	0/4	200	2/4	230	1/4
	675	2/4	675	4/4	852	2/4	852	3/4	300	1/4	300	4/4
	1,012	4/4	1,012	4/4	1,125	4/4	1,125	4/4	450	3/4	393	4/4
LD_{50}: (mg./Kg.)	675		498		650		740		300		244	
95% confidence range of LD_{50}:	534 to 853		406 to 610		558 to 756		650 to 845		183 to 491		213 to 279	

All bisulfite deaths occurred no later than 45 minutes post-injection, a period characterized by extreme irritability with clonic convulsions, respiratory instability (including apneic and cyanotic episodes), evidence of cardiovascular collapse and protracted apnea. All other animals were observed for 72 hours post-treatment. Mice were Swiss-Webster white, rats were Sprague-Dawley white, rabbits were New Zealand white and the dogs were mongrel.

2. Ref. 111 (sodium sulfite)

	Animal	Sex/no.	Route	Dose(mg/kg)	Measurement
(a)	rabbit	?/1	i.v.	580	ED
(b)	"	"	"	1000	LD
(c)	"	"	"	760	LD
(d)	"	"	"	630	ED
(e)	"	"	"	690	LD
(f)	"	"	"	670	ED
(g)	"	"	"	680	ED
(h)	"	"	"	710	LD

(a) A 1.5 kg rabbit received 7 ml of 1.0 N sodium sulfite i.v. over the course of 12 min. The pulse was strong but very slow. The injection was terminated at cardiac arrest. The animal rapidly revived, later accepted food and remained well.

(b) A 1.5 kg rabbit received 12 ml. of sulfite over 33 min. At 5 ml convulsive shock was followed by a one-minute suspension of injection; the heart remained strong. At 10 ml. the heart was still strong but an accentuated slowing of the pulse led to a 5 min. suspension of injection. At termination the released animal was moribund, exhibited sporadic and shallow respiration and died in 5 min.

Autopsy revealed brownish-red lungs; flaccid heart, stopped in diastole; liver, kidneys and other viscera normal.

Histology:

kidney - congestion of glomeruli

lung - alveolar and bronchiolar extravasation; erythrocytes undeformed

myocardium - grossly normal but with evidence of extravasation of fibers

liver - parenchymal and hepatic cells normal

(c) A 1.4 kg rabbit received 8.4 ml sulfite; at 5.5 ml, the pulse declined to 160; respiration, 52; at 7 ml (13 min), convulsions; pulse 96 with arrhythmia; tachypnea, shallow respiration; at termination (31 min), clonic convulsions shortly after; and death.

Autopsy revealed a flaccid heart, arrested in diastole; brownish-red lungs; kidneys normal in shape and texture with slightly hyperemic cortex and pallid medulla; hepatomegaly with some coccidial nodules; gall bladder enlarged with pallid bile; bladder full of urine clouded by bile.

Histology:

kidney - as in (b)

lungs - as in (b)

myocardium - no changes in fiber; some slight capillary hemorrhage

liver - normal

(d) A 1.2 kg rabbit receiving 6 ml over 30 min displayed the usual dyspneic, convulsive and cardiac symptoms but recovered well at termination.

(e) A 1.45 kg rabbit received 8 ml over 30 min. with the usual symptoms. The released animal was very depressed and lay down on its side; respiration, 60; after 15 min, leg paralysis; then full paralysis and death within 25 min.

Autopsy was essentially similar to (d).

Histology:

kidney - congestion of the medullary and cortical capillaries; normal tubular epithelia

lungs - alveolar and bronchiolar infarcts with erythrocyte extravasation

myocardium - normal

liver - normal

(f) A 1.4 kg rabbit received 7.4 ml over 30 min. At 5 ml, the animal was calm with strong heartbeat but some dyspnea; at 7 ml, pulse slowed to 120 with arrhythmia; at termination the animal quickly recovered.

(g) A 1.5 kg rabbit receiving 8.1 ml over 45 min. showed only dyspnea and a slowing of the pulse, no arrhythmia; it recovered well.

(h) A 1.0 kg rabbit received 5.6 ml over 60 min. Dyspnea and slow pulse were evident at 2.4 ml; pulse, 64 at 5 ml, with arrhythmia; pulse, 33 at termination, with leg paralysis and, after 10 min even posterior paralysis. It revived to some extent but then respiration became labored and tonic-clonic leg contractions began; exitus letalis within 35 min.

Autopsy showed a trace of pleural cavity fluid; normal lungs; heart flaccid, in diastole; normal liver; and slightly congested renal cortex.

Histology:

kidneys - as in (e)

lungs - as in (e)

myocardium - normal fibers; recent capillary hemorrhage

liver - normal

Thus death is attributed to simultaneous action on the respiratory and circulatory centers. Anatomical and histopathological study revealed no serious lesions. Moreover, the in vitro hemolytic action of sulfite is not implicated.

3. Ref. 69 (sulfur dioxide)

animal	strain	sex/no.	route	dose(mg/kg)	measurement
rat	Wistar	?	p.o.	1040*	LD_{50}
"	"	"	"	2000**	LD_{50}
"	"	"	"	1560***	LD_{50}

* as a 6.5% aqu. solution
** as a 3.5% aqu. solution
*** for the acetaldehyde adduct

4. Ref. 65 (sodium bisulfite)

animal	strain	sex/no.	route	dose(mg/kg)	measurement
rabbit	chinchilla	?/15	i.v.	65	LD_{50}
hamster	golden Syrian	?/30	"	95	"
rat	Sherman	?/30	"	115	"
mouse	Webster albino	?/30	"	130	"

Symptoms preceeding death were the same in all species: almost immediate respiratory depression; terminal clonic convulsions and death following respiratory arrest ten to twenty minutes post-injection. No delayed deaths occurred.

5. Ref. 65 (sodium sulfite)

animal	strain	sex/no.	route	dose(mg/kg)	measurement
mouse	Webster albino	?/30	i.v.	175	LD_{50}

The clinical picture was the same as with sodium bisulfite in No. 4 (above).

6. Ref. 80 (potassium metabisulfite)

animal	strain	sex/no.	route	dose(mg/kg)	measurement
rat	Wistar CF	M/30	p.o.	1500	LD_{29}
"	"	"	"	2000	LD_{57}
"	"	"	"	2500	LD_{85}
"	"	--	--	1000	LD_0*
"	"	--	--	1800	LD_{50}*
"	"	--	--	3600	LD_{100}*
"	"	--	--	580	LD_0**
"	"	--	--	1040	LD_{50}**
"	"	--	--	2060	LD_{100}**

* interpolated or extrapolated estimation

** estimation for SO_2 based on % available SO_2 in $K_2S_2O_5$

Rats of a mean 250g body weight, 2 months of age, received aqueous metabisulfite by intubation. Post-treatment trauma was biphasic within 5-20 minutes: first, a depressed behavior with prostration and generalized and generalized anesthesia, second, gastric spasms, convulsions, tetanization and death by cardiac arrest. Histopathology found generalized tissue plethora without evidence of specific lesion.

7. Ref. 114 (sodium bisulfite)

animal	sex/no.	route	dose(mg/kg)	measurement
rabbit	?/6	i.v.	106	MLD [sic]
mouse	?	i.p.	750	LD_{100}
"	?/5	"	500	LD_{40}
"	?/4	"	250	LD_0

Lethal doses of aqueous i.v. (ear vein) sodium bisulfite in mature white rabbits exerted an instaneous CNS effect with clonic convulsions and a cardiovascular effect-rapid fall of blood pressure-followed by death in a few minutes, due probably to blockage of oxidative mechanisms. Histopathology of heart, lung, liver, kidney and spleen was negative.

Albino mice, 22-25g, were injected i.p. with 0.1-0.5 ml of 0.6M sodium bisulfite. All animals at the 0.3-0.5 ml level died within a few minutes. Two of five at the 0.2 ml level died soon after injection, the remaining three surviving. All four at the lowest level survived.

II. Short-term studies

1. Ref. 66 (postassium metabisulfite)

Method

Species: rat
strain: Wistar
sex: M
age at start of experiment: 30 days
duration of study: 10 days
vehicle: in diet
dose schedule: 0.5 or 1.0%
route of administration: p.o.
number of animals per level: 8

Discussion

Either 0.5 or 1% dietary potassium metabisulfite significantly increased urinary, but not fecal, calcium at normal 0.5% dietary calcium levels. Fecal, but not urinary, calcium excretion after 1% ingestion was significantly increased by both 0.5 and 1.0% metabisulfite. There was no effective difference between 0.5 and 1% metabisulfite on calcium turnover.

Two mechanisms are suggested. Since renal excretion of calcium is difficult in the rat, a gradient might be reached by 0.5% metabisulfite which would tend to depress the effect of higher metabisulfite concentrations. Calcium at the 1% level would become excess calcium for clonic delivery; this would explain the elevated fecal elimination at the higher calcium diet. The absence of effective differences between low and high metabisulfite levels could also follow from a rate limiting step in sulfite-sulfate oxidation.

2. Ref. 18 (sodium metabisulfite)

Rats fed on a diet supplemented with 0.6% sodium metabisulfite showed loss of appetite after 3 weeks. Later apathy, bradycardia and clinical polyneuritis developed. This was found to be due to deficiency of thiamine, which is destroyed by metabisulfite. Intramuscular injection of thiamine restored growth.

The storage of sulfied diets at room temperature led to slow development of additional toxicity, not attributable to thiamine deficiency. Its cause is unknown.

3. Ref. 33 (sulfur dioxide)

Grape juice was given ad lib to 80 mice and rats for 6 months daily as liquid supplement to an unspecified diet. Concentrations of 100 ppm SO_2 and 50 ppm SO_2 were used.

There were insignificant tissue changes after 4 months at the higher dose. Thoracic and cervical abscesses, leukopenia, eosinopenia, displacement of adrenal ascorbic acid and reduced liver glycogen were found in rats at 5 months. At 2 months the mice lost significant weight and were sluggish, disorderly and diarrheic. Electromelia was found by month 5 and all animals succumbed by the 6th month. Equivalent control groups were unaffected.

Histopathology of both groups of animals included subpleural hemorrhages, plethora and marked gastric distention with edematous and hemorrhagic mucosa. Gastric gland epithelial desquamation, involving the entire cell layer at some sites, necrosis and lymphocyte-histiocyte infiltration of stroma was also marked. In the mouse there were cell infiltrates randomly distributed in the mucosal and muscle stroma extending up to esophageal levels with pharyngeal desquamation. Both mouse and rat hepatocytes showed turbid swelling due to albuminoid granulation and detachment with diminished nuclei or anuclear cells. Cells near the central hepatic vein, whose lumen was dilated, exhibited nuclear extrusion and plasmatorrhexis. In rat liver cell infiltrates with round lymphoid and histiocytic types

displaced atrophying hepatocytes. Cytoplasmic vacuolization of medullary cells and enlargement of the zona glomerulosa and fasiculata were found in the adrenal.

At the lower SO_2 dose (50 ppm) oxygen consumption increased during the first 3 months and liver glycogen fell in rats. In mice there was weight loss by month 3 and increased gaseous exchange by month 5. Macroscopically, visceral plethora and edematous and hyperemic gastric mucosa was characteristic of both animal groups. The histopathology was the same as that following 100 ppm but less acute. Control mice showed some weight loss by month 3, and control rats by month 5 but no other gross changes were found, except for a slight plethora. Histologically, there was insignificant epithelial desquamation of gastric mucosa.

Ascorbic acid in the adrenals was increased 60 and 30%, liver glycogen was decreased 56 and 37%, alkaline phosphatase activity fell 48 and 34% and serum oxidase activity declined 21 and 12%, resp., after the higher and lower doses.

4. Ref. 65 (sodium bisulfite)

Method

Species: rabbit

strain: chinchilla

age at start of experiment: ? wt.- 2.5 kg

duration of study: 3 weeks

vehicle: dist. water or 6% Parenamine (an acid hydrolyzate of casein)

dose schedule:

1. in distilled water
   - (a) 10 mg/kg three times daily
   - (b) 20 " " " "
   - (c) 40 " " " "

2. in Parenamine
   - (a) 10 mg/kg three times daily
   - (b) 20 " " " "
   - (c) 40 " " " "

route: i.v.

number per level: 3

Observations

Symptology: A severe vascular irritation leading to thrombosis in ear veins at injection site in group 1. only; no symptoms of acute or cumulative toxicity were evident in either group

weight change: increases of from 8-23%, about the same as controls

survival: all till sacrifice of groups (c) at termination

gross pathology: no gross abnormalities

microscopic pathology: no histopathology in heart, lungs, liver, spleen or kidneys

Discussion

The authors point out that a daily, divided dose of nearly twice the LD_{50} could be administered for three weeks with no apparent toxic outcome.

The thrombi found in group 1. were ascribed to the presence of free SO_2.

5. Ref. 125 (sodium bisulfite)

Method:

Species: mouse

strain: cross-bred white

sex: both

age at start of experiment: young (8-10g)

duration of study: 3 months

dose schedule: 160 mg/kg/day

route of administration: p.o. (intubation)

no. of animals per level: 100 (50M, 50F)

Discussion:

A daily 3-month intake of 160 mg/kg sodium bisulfite led to a 143 and 124% gain over initial body weight, resp., for males and females. The present survival was 70% for males and 68% for females. A subgroup of 10 mice fed the 160 mg/kg dose for 2-3 months were each given a single dose of 0.1 ml carbon tetrachloride by intubation after termination of bisulfite feeding. The 29% mortality of control mice rose to 45%. Another group of 14 mice on 160 mg/kg $NaHSO_3$ was placed under starvation conditions of a 90% food restriction immediately on termina-

tion of the 3-month feeding; there was a 37% weight loss and a 57% mortality after only 5 days fasting, with a 4.6 day survival for the remaining animals. Bisulfite fed as a paste directly before the main feed had no effect on daily food or water intake.

6. Ref. 69 (sulfur dioxide)

Method

Species: rat
strain: Wistar
duration of study: 12 months
vehicle: aqueous
route: p.o.

Discussion

Rats weighing 400 g were given 10 ml daily of aqueous SO_2 (1200 ppm) for 5 months (30 mg/kg) and then 10 ml daily of aqueous SO_2 (6000 ppm) for 7 months (150 mg/kg). No toxicity was observed other than a decrease in oxidations in hepatic cells, and this only with free SO_2.

7. Ref. 138 (sodium bisulfite)

Male rats were fed a daily diet containing 1600 mg/kg of sodium bisulfite and 0.2 mg thiamine per 100g feed. All animals had died by the end of the twelfth week of experimentation.

8. Ref. 103 (sulfur dioxide)

Exposure of albino mice to 0.15 ppm SO_2 for 3 months and 0.23 ppm for 6 weeks produced remarkable congestion and hemorrhage of the lungs. Thickening of smooth muscle cells of the blood vessels and bronchi were observed in the former group. One quarter of each group died from exposure before the end of the experiment and these and several other mice possessed multiple abscesses of the lung. Histology also revealed slight congestion and hypertrophy of splenic lymph follicles and inflammatory cell infiltration of heart and liver.

9. Ref. 45 (sodium bisulfite)

Method

Species: rat
strain: Osborne-Mendel
duration of study: up to 52 weeks

vehicle: in diet

dose schedule: see discussion

Discussion

One group of animals received 0.5, 1.0 and 2.0% sodium bisulfite in a stock diet, supplemented in some cases by i.m. thiamine injection, for up to 52 weeks. A trend toward reduced weight gain and lesser average weights was noted at all dose levels.

Another group of rats was given 0.1 and 0.25%, and a third received 0.0125, 0.025 and 0.05%, sodium bisulfite. Diets with 0.05 and 0.1% led to greater weight gain and faster rate of gain than that for control animals during the first 12 weeks. At 52 weeks the gain on 0.1% had fallen significantly below control levels, while the 0.05% group was within control limits. No difference was found with thiamine supplementation. No trends in survival time could be associated with diets below 0.1%. In every case a 2% diet was correlated with a shorter average survival, while 0.1 and 0.25% diets showed trends in the same direction. No difference was found with thiamine supplementation, but thiamine depletion markedly reduced survival time.

No significant pathology was found with dietary levels below 0.25%, except a diarrhea specific for sodium bisulfite at 0.1% or more. The type of injury was relatively invariant for diets between 0.25 and 2%, although survival time was longer and pathogenesis slower. Higher bisulfite doses often led to clinical polyneuritis, bleached incisors, uterine browning, visceral organ atrophy, calcified renal tubular casts, bone marrow and bone atrophy, growth stunting and spectacle eyes. Hyperplasia of gastric squamous epithelium and focal myocardial fibrosis, chiefly of the left heart, were generally moderate and less frequent than the above-cited. Fatty liver degeneration was negligible, with highest frequencies in control, sulfate or sulfide diets. Little tubular atrophy followed renal calcification. Intertubular edema in in testes occurred at the 2% level. Bone and marrow changes were unremarkable and attributed to inanition. Lung, adrenal, pancreas, small and large gut, ovary, spleen, thyroid, brain and sciatic nerve were histologically negative.

10. Ref. 85 (sulfur dioxide and sodium metabisulfite)

Method

Species: cow

strain: (a) Jersey; (b) Holstein

sex: steers

age at start of experiment: (a) 14 mos.; (b) 9 mos.

duration of study: (a) 16 days; (b) 21 days

route: rumen fistula

dose schedule: see discussion

number: one of each

Observations

Steer (a) was treated with SO_2 gas introduced into the space above the rumen liquid via a rumen fistula; 50 g/day for one week, 162 g/day for week two and 255 g/day for two days of week three when the animal died. The total dose was 1994 g or ca. 7300 mg/kg based on initial body weight.

Body temperature on the fifth day was 104.8°F and anorexia became evident on day ten. A heavy, mucopurulent nasal discharge, followed by diarrhea, constipation and coughing, began on day nine and continued till death.

Due to the occurrence of considerable dorsal rumen wall necrosis in steer (a), the gas was introduced directly into the rumen fluid in (b). The dose schedule was 53 g/day during week one; week two, 73 g/day; week three, 145 g/day; and 47 g the next day when death occurred. The total dose was 7200 mg/kg.

Anorexia commenced on day fourteen. Other signs were similar to (a), except for terminal nystagmus and severe depression.

Hematology: A general, but variable increase in hematocrit values, related to the anorexia, was noted. Erythrocyte counts paralled hematocrit and Hb decreased gradually with minor fluctuations. Leukocyte counts averaged 9500-15,000/mm^3 with several one or two-day peaks of 21-35,000 toward the end. Icterus index was 10-15 during the last 5-10 days. At the higher dosages neutrophils peaked irregularly, with a corresponding shift of large and

small lymphocytes. Apart from a few small to moderate single day increases in eosinophils, monocytes and basophils there were no significant changes in these parameters.

Biochemistry: Blood glucose showed no significant variation, but blood urea nitrogen dropped in both cases.

Necropsy: The rumen wall was thickened; there was mild subserous suffusion hemorrhaging and moderate mucosal congestion. Microscopic evidence of severe congestion and edema of the lamina propria of the congested portion of the rumen was evident. There were a few localized hemorrhages and erosion and leukocyte infiltration of the papillar tips. The reticulum was congested, again with erosion and leukocyte infiltration of papillar tips. The omasum was grossly and microscopically similar.

One animal had acute hemorrhagic gastritis of the abomasum with numerous bleeding points and a chocolate-colored abomasal fluid. There was histologic evidence of erosion, leukocytic infiltration, congestion and mucosal hemorrhage.

Animal (a) showed a few petechia and marked catarrhal enteritis of the small intestine; (b) had hemorrhagic duodenal enteritis which "changed into" catarrhal enteritis. Histopathology showed hyperemia, edema, swollen villi and cellular infiltration of the propria.

Animal (b) also had acute catarrhal enteritis of the colon. Microscopic evidence again indicated superficial desquamation, erosion, hyperemia and occasional petechiae.

The liver of (b) demonstrated coagulation necrosis, cloudy swelling and moderate fatty degeneration, while the gall bladder had several ecchymotic mucosal hemorrhages and also thickened bile. There were also petechiae of the spleen.

The kidneys of both were moderately to severely nephrotic with cystic dilatation of the convoluted tubules and occasional leukocytic infiltration.

Steer (b) had severe petechial ecchymotic and suffusion hemorrhages of the ventral wall of the cranial portion of the trachea, and a moderate number of these hemorrhages in the myo- and endocardia. Submucosal edema of the trachea was also evident.

There were mild congestion and edema of the leptomeninges with an apparent increase in cerebrospinal fluid. Some inflammation of the leptomeninges was microscopically observed.

Discussion

The results of this study emphasize the systemic, as well as localized effects of SO_2 administration.

Anorexia and weight loss were the principal signs of intoxication.

The most severe lesions were generally confined to the first part of the digestive tract, i.e. a localized effect at the site of administration, while the tracheal lesions may have been due to condensation of inspired rumen gas.

The drop in blood urea suggests to the authors a disturbance of nitrogen balance.

It had been assumed that SO_2 would exert a depressing effect on rumen microflora, but a cow receiving 80 g/day of sodium metabisulfite for six months remained apparently normal.

Another cow, in her third months of pregnancy, received the above dosage for 180 days and calved normally with no aberration of the mother or calf.

III. Long-term studies

1. Ref. 34 (potassium metabisulfite/sulfur dioxide)

Method

Species: rat

Strain: Wistar

Sex: both

Age at start of experiment: 28-32 days

Duration of study: 20 months

Vehicle: distilled water

Dose schedule: see Discussion

Route of administration: p.o. (in drinking water)

Number of animals per level: 80

Discussion

The amounts of $K_2S_2O_5$ and SO_2 ingested were as follows:

Interval	mg/kg of ration consumed				mg/kg of body weight			
	$K_2S_2O_5$		SO_2		$K_2S_2O_5$		SO_2	
	M	F	M	F	M	F	M	F
2 weeks	1630	1780	950	1030	330	330	190	190
2 months	1490	1890	860	1100	100	135	60	80
9 months	1570	1870	910	1080	61	93	35	54
20 months	-	-	-	-	50	70	30	40

Body weight increase for either sex varied only insignificantly between control and experimental animals at all test intervals. Nor was there significant change in organ weights (heart, liver, kidneys, adrenals, gonads), except a 29% splenomegaly in test females. Mortality differences adjusted to the results of a colony-wide respiratory infection were found to be insignificant.

There was no difference between control or test reproductive rates or outcomes through F_2, except for a 19 and 10% reduction, resp., in the F_1 and F_2 of metabisulfite-treated F_0 females. There were also significantly fewer F_1 and F_2 males born in the treated group.

Macroscopically, massive subcutaneous tumors were found, especially at abdominal levels, but the frequency was equally distributed over both control and test groups. The histological statistics of pulmonary abcess,

eosphageal injury and renal lesion were similar for both groups. Hepatic steatosis and sinusoidal dilation, especially centrolobular, had a high frequency in both groups. Slight evidence of histologically unremarkable superficial mucus cell necrosis in each group was attributed to artifact. The erythrocyte number was unchanged in both test and control groups. A leukocytosis of 20 and 10% was found for treated males and females, resp., with neutrophilia typically associated with pulmonary insult. There was also a 0.5 and 3% eosinopenia, resp., for test males and females and a 2% monocytopenia in treated females only.

2. Ref. 84 (sodium metabisulfite)

Sodium metabisulfite was added to the drinking water of 54 rats (uniform strain bred for cancer research) on stock diets for 2 1/2 years, or 3 generations. Metabisulfite concentrations equivalent to 350 and 750 ppm SO_2 were used. No difference was found between the growth rates of control and experimental animals at either dose. There was no significant difference in spontaneous food intake, although growth rate and food consumption tended to increase in experimental F_2 and F_3, especially among females. F_3 females also evidenced a higher fecal excretion (or, faster than normal intestinal passage); there was no other difference between test and control groups for any generation.

Based on litter matings at each generation, metabisulfite had no effect on fertility, postnatal survival, lactation behavior or general health. Post mortem examination confirmed no significant difference in body weight or organ weight between test and control rats, even at 750 ppm SO_2 for 2 1/2 years. Polyneuritis, spectacle eyes, incisor blanching or uterine browning were not detected. Histology was routinely negative. The carcinogenesis rate was 20/54 and, although higher among females, could not be attributed to the SO_2. Adiposity occurred in 27/54, but was equally distributed among test and control animals. Gastric mucosal hyperplasia was entirely absent.

3. Ref. 67 (sodium bisulfite)

Eight groups of 18-24 Osborne-Mendel rats were given 0.0125, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0 or 1.5% sodium bisulfite in basic diets of mainly cornstarch and casein composition, starting on the 21st day of life and continuing for 1-2 years. Incisor development in 43 rats was the target system. Pigmentation deficit was dose-dependent and was detected variously in 2/3 of the experimental animals. Atrophy of the enamel organ was atypical and premature, a classical index of vitamin E deficiency. Dentin formation was grossly reduced and atypical at 1% bisulfite levels, with dysfunction and atrophy of the odontogenic epithelium. Of the incisor support structures, the lingual periodontal membrane, alveolar bone and lingual gingiva all showed degrees of abnormality. The respiratory epithelium of the nasolacrimal duct was replaced by stratified squamous epithelium at 1% levels, above which the keratinization varied directly with dose, a specific sign of vitamin A deficit. It is proposed that bisulfite degrades vitamin E, thus exposing vitamin A to oxidation and elimination.

4. Ref. 3 (sulfur dioxide)

Cynomolgus monkeys exposed to concentrations of 0.14, 0.64 or 1.28 ppm SO_2 for 78 weeks showed no significant variation from a control group in body or organ weight, survival, mechanical properties of the lung, hematological properties, lymphocyte or neutrophile concentration, total urea nitrogen of the blood or histological changes in the lung. A group exposed for 30 weeks to 4.69 ppm and then 1000 ppm (accidental) for 1 hour showed significant alteration of lung and liver tissues, e.g., alveolitis and bronchiolitis with pneumocyte hyperplasia in lung and focal hepatocyte vacuolation of liver.

5. Ref. 69 (sulfur dioxide)

Four successive generations of Wistar rats over the course of two years were given water or wine containing 450 ppm SO_2, 3 ml per day per 100 g of body weight (13.5 mg/kg). No toxicity other than a small diminution of cellular oxidation in hepatic tissue.

6. Ref. 125 (sodium bisulfite)

Method

Species: (A) mouse; (B) rat

strain: (A) cross-bred white; (B) Wistar

sex: both

duration of study: (A) 17 months; (B) 18 months

dose schedule: 80 mg/kg/day

route: in diet

no. per level: (A) 50; (B) 20

Discussion

There was no mortality in a group of 17 animals during a 5-day interval of fasting following 17 months exposure to regimen (A). Although there was an average 18% weight loss, only 2 1/3 days were subsequently required for compensation. Relative liver, kidney and testicular weights at sacrifice were unremarkable.

There was a slight reduction in daily feed and water intake for male, but not for female rats in (B). Both 430 and 860 mg/kg $NaHSO_3$ given to control rats at the end of the 18 month feeding study led to 100% mortality. An LD_{100} also followed administration of 860 mg/kg to rats at the end of 18 months of a combined bisulfite-benzoic acid diet; a 430 mg/kg dose given to the same group elicited only on LD_{60}, showing some suggestion of tolerance.

IV. Special Studies

A. Carcinogenicity

1. Ref. 41 (sodium bisulfite)

Ehrlich ascites carcinoma was transplanted i.p. in 97 mice of both sexes after a 3 months stock and briquette diet containing 0.4% sodium bisulfite. Tumor development was followed for 53 days. Tumor incidence was 61/97, or 63%, a highly significant outcome about twice that for controls. Survival time, defined as the time at which the mice began to die, was 12 days, or half the control value. Mean ascitic fluid volume (6.5 ml) was significantly higher than that either for controls or other experimental animals on different additive regimens. Blood content of the fluid, as measured qualitatively in degree of hemorrhagic units, was insignificantly higher than controls. The tumorigenic effect is thought to be independent of any tendency toward adiposity in the treated group.

2. Ref. 125 (sodium bisulfite)

Ehrlich ascites mouse carcinoma was transplanted i.p. in mice at termination of a 3 month sodium bisulfite regimen of 80 mg/kg per day by intubation. Tumor growth was higher in this group than that in animals receiving either bisulfite/benzoic acid combined diets or benzoic acid alone.

B. Reproductive effects

1. Ref. 7 (sulfur dioxide)

Slight blastoderm retardation and complete absence of embryogenesis followed a 2-hour exposure of a chick embryo system to SO_2. The same effect was elicited by 3 minutes of H_2S or ammonia, 2 hours HCl, 5 hours chlorine, 2 days commercial acetylene, 3 days CO_2 or 6 days methane ("illuminating gas"). Carbon monoxide had no effect on embryogenesis despite 8 days exposure.

2. Ref. 132 (sulfur dioxide)

Progeny from sows fed daily with 3 kg mash containing 1660 mg SO_2 during the second half of pregnancy died from septic gastric and intestinal inflammation. Eight pigs receiving 2 kg mash (1130 mg SO_2) daily for 1 month exhibited a low daily weight gain and poor feed utilization. A cow on 6 kg mash (653 mg free and 4270 mg total SO_2) for 3 weeks gave only 8 liters of milk daily, or 14 liters below normal.

C. Mutagenicity

1. Ref. 124 (sodium bisulfite)

$NaHSO_3$ adds reversibly to the 5,6-double bonds of both cytosine and uracil under physiological conditions. Upon standing the cytosine-HSO_3^- addition product deaminates to the uracil-HSO_3^- compound. Upon adjustment of the pH to 10 this addition product decomposes completely back to uracil and HSO_3^-. This is a specific method for deamination of cytosine to uracil, since neither adenine nor guanine react with $NaHSO_3$ and the addition to uracil is reversible.

2. Ref. 96 (sodium bisulfite)

A reversion of the genetic code was found when a CG mutant A^{38} arg^- strain of E. coli was suspended in 1M $NaHSO_3$ at pH 5.2 for 30 min. 19 AT mutants, 3 deletion mutants and four UAG amber mutants did not show reversion. It was concluded that $NaHSO_3$ is specific for CG mutants and causes back-mutation by deamination of cytosine to uracil.

3. Ref. 59 (sodium bisulfite)

Mutation at the c genes of lambda phage was attributed to sodium bisulfite conversion of 5-methylcytosine to thymine. Adenine and guanine were unreactive. The mutation frequency was a function of time, with a maximum of 107 mutants in 40,800 plaques screened at 1 1/2 hours. Longer treatment did not increase the frequency. Scalar indices show bisulfite to be somewhat more mutagenic than hydroxylamine.

BIOCHEMICAL ASPECTS

I. Breakdown

1. Ref. 117

The application of SO_2 in the preservation of dried fruit to be used for making "artificial" wines, mince meat or sauces is questioned. Yeast fermentation is possible in diluted mixtures and SO_2 is partly reduced to hydrogen sulfide (toxic) in objectionable concentrations.

2. Ref. 14

Orange oil, in the presence of SO_2 (as sodium metabisulfite), was partially oxidized within 4 weeks when stored at 35°C. Orange oil, without SO_2 added, showed no obvious oxidation under identical conditions. Alkene and carbonyl components and the SO_2 itself were oxidized, with the uptake of one mole of O_2 for each mole of SO_2 added. p-Cymene, carvone, carveol, α-terpineol and other unidentified products were present in the oxidized oil.

II. Absorption-Distribution

1. Ref. 142 (sulfur dioxide)

The upper airways of anesthetized dogs were exposed to an atmosphere of 20-50 ppm $^{35}SO_2$ for 30-60 min. and the distribution of the label was followed for several hours after exposure. At the peak 5-18% of the label was in the blood and it was always more concentrated in the plasma than in the red blood cells. One third of the plasma activity was associated with plasma proteins and two thirds of the activity of the red blood cells was intracellular. Most, about 84%, of the sulfur in the urine was sulfate; most of the blood sulfur was presumed to be sulfate, also.

III. Metabolism and Excretion

1. Ref. 19 (sodium metabisulfite)

Sodium metabisulfite solution (2000 ppm as SO_2) was given p.o. to 4 rats (148 g) for 3 days, with total food restriction the final 24 hours and a subsequent amount of solution equivalent to 5% of weight by gavage. Metabisulfite load in the 4-hour post-treatment urine was 9.3 ml; inorganic sulfur as $SO_4^=$ amounted to 33 mg, reducing power $SO_3^=$ and organic sulfur as $SO_4^=$ were equivalent, resp., to 2 mg and 0.4 units. Since the water load in urine volume was 8.1 ml, 53% of the mean oral metabisulfite was excreted in the first 4 hours. The inorganic sulfur as $SO_4^=$ in the water was ca. 11 mg; therefore, the total sulfate equivalent of metabisulfite input per rat was ca. 44 mg. Since there was only trace evidence of organic sulfur as $SO_4^=$, or $SO_3^=$ reduction of iodine, inorganic sulfate must have been the main component excreted. In another experiment designed by a 4 x 4 latin square for 12 rats (261 g), the animals were fasted for 18 hours before receiving a volume of water equivalent to 2 1/2% body weight. One hour later various test solutions equivalent to 5% body weight, including sodium metabisulfite and sodium thiosulfate, were given p.o. and the 4-hour urine collected. The mean sulfur loads after metabisulfate and thiosulfate ingestion, resp., were 55 and 23%, while the water load for each was 66 and 45%. Metabisulfite was excreted mainly as inorganic sulfate (197 mg), with only 2% organic sulfate. Thiosulfate was also recovered mainly as inorganic sulfate (79 mg), with a 4% etheral sulfate excretion.

A third study simulated the initial conditions of the preceding 2 experiments except for a random block design and i.p. administration of isotonic sodium metabisulfite and thiosulfate in volume equivalent to 3% body weight. Signs of restlessness were followed directly by cyanosis, prostration and cardiovascular collapse in all 12 animals after metabisulfite treatment. An LD_{50} was reached 35 minutes post-injection. The surviving 6 animals showed signs of recovery at 40 minutes with normalization 30 minutes later. Thiosulfate elicited no adverse effect. The urinary pattern after one-twelfth of the LD_{50} was given i.p. and confirmed that neither the form nor amount of sulfur excretion differed between metabisulfite and thiosulfate. In the 4-hour urine 80-90% had been eliminated as inorganic sulfate. It is concluded that, although

the rates of metabisulfite and thiosulfate absorption from the gastrointestinal tract differ, both anions are subject to the same hepatic oxidation to sulfate.

2. Ref. 137 (sodium bisulfite)

Continuous splenic vein infusion of 160 mg/kg $NaHSO_3$ per hour for 3 hours in 3 pentobarbital-anesthetized dogs with ureteral ligature was immediately followed by a 3-hour infusion of 80 mg/kg $NaHSO_3$ per hour. Hourly samples were drawn from the femoral artery. Levels of 50-140 µg bisulfite, which represent modest increases, were found per ml serum after the 160 mg/Kg rate. Halving the infusion rate dropped serum levels to 25-50 µg/ml. This would suggest a minimum bisulfite to sulfate oxidation rate of 80 mg/Kg per hour, a value said to be 4x the maximum conceivable exposure in humans with renal embarrassment undergoing chronic intermittent peritoneal dialysis.

IV. Effect on enzymes and other biochemical parameters

1. Ref. 42 (sodium bisulfite)

Sodium bisulfite was tested on the saliva of 22 human patients in vivo and in vitro and was found in all cases to markedly inhibit acid formation. The mode of action was thought to involve reaction with aldehydes from carbohydrate metabolism and consequent inhibition of lactic acid formation.

2. Ref. 39 (sodium bisulfite)

The chemotactic property of polymorphonuclear (PMN) exudate cells could be inhibited in vitro by M/8 $NaHSO_3$. The anion has no effect on the complement or calcium ions, whose presence represents the sufficient conditions for chemotaxis. It is inferred that bisulfite acts directly on neutrophil enzyme assembly, possibily interfering with the surplus peroxidase in PMN's which has been related to increased cell mobility.

3. Ref. 74 (sulfur dioxide)

Samples of wine (free SO_2 equal to 22 mg/l) and SO_2 concentrations (300 mg free sulfurous acid) in drinking fluid by intubation were given to rabbits (2.2-2.6 kg) on standard diets. Blood was drawn from ear veins at different intervals and either assayed directly or added to staphylococcal cultures for 4 hours incubation. A pre-treatment control value of 62% decrease in staphylococci density rose to 86% 24 hours and 88% 48 hours after a single SO_2 dose.

The mean of 88% was sustained up through post-treatment day 5. Differences between wine and SO_2 effect were negligible, and both were considered insignificantly different than controls. Complement fixation with SO_2 rose ca. 25% 6 hours post-treatment and fell to normal by 48 hours, where it remained for 5 days; the titration values are expressed as reciprocals. In another study with a daily dose of 5 SO_2 administrations, the titer reciprocal rose 25% by 1 day and remained at that level for another 24 hours.

The differential blood picture after single doses of either SO_2 or wine found no substantial change in erythrocytes and only a slight tendency toward leukopenia by post-treatment day 5. There was a slight neutropenia by hour 6 and a slight neutrophilia by day 5 for SO_2 but an increasing neutrophilia for wine up to day 5. An eosinopenia was detected after single SO_2 doses between 3 and 24 hours and a slight increase at 48 hours, with normalization by day 5. A single administration of wine led to an eosinophilia within 48 hours and eosinopenia by day 5. Lymphocytes were increased ca. 20% by SO_2 within 48 hours, with a compensatory lymphocytopenia by day 5. Increasing lymphocytopenia from 1 hour to 5 days post-treatment followed wine exposure. Monocytes and basophils tended to decline up to day 5 after SO_2. There was a tendency toward basophilia increasing with time, after wine ingestion, with only a modest monocytopenia between 6 and 48 hours. The picture following 5 SO_2 administrations in one day remained unchanged , excepting for a basophilia between hours 24 and 48.

4. Ref. 23 (sulfur dioxide)

Using a special experimental design, 25 ml of human blood taken with dextrose citric acid was able to convert 3 ml SO_2 (7.95 mg), liberating 0.03 mg. Additional studies were made on SO_2 compartmentalization between plasma and cells, injecting 2.65 mg SO_2 in plasma and 5.3 mg in the formed elements after centrifugation of 25 ml blood. It was found that twice as much SO_2 was converted by the cells as by plasma; however, the percent SO_2 changed was not identical for the same blood sample if the results obtained on whole blood are compared to those obtained separately on plasma and cells. SO_2 fixation in blood containing 100% carboxyhemoglobin was significantly less than that for normal blood; in this case, the effect of cell elements was greatly reduced by that of plasma remained unchanged. There was no difference in reactivity between normal and 25, 50 or 75% oxygenated blood. Loss of secondary, tertiary and quaternary

protein structure in denatured plasma or erythrocyte stroma had no effect on rate of SO_2 fixation. Complete alcohol deproteination of plasma also was without effect. Changes in fixation elicited by Cu^{++}, Cu^{++} and Hg^{++}, Hg^{++} and urea and Hg^{++} and guanidine were insignificant.

5. Ref. 105 (sulfur dioxide)

Anhydrous SO_2 was pumped for 1 hour at 250 ml/min through a 60 ml enzyme solution of 6μM bovine erythrocyte (Type I) acetylcholinesterase. Above 4 ppm the relative enzymatic activity plotted against SO_2 concentration gave an exponential curve of general form $y = 100 - be^{x}$, where y is the relative enzyme activity and x is the SO_2 concentration. The b value for SO_2 was 2.5. Relative enzyme activity fell nearly 50% at 10 ppm, and the pH dropped almost 2 units from a pre-treatment pH of 6.8. Sodium bisulfite was a weaker enzyme inhibitor than SO_2, since its effect was equivalent to a final SO_2 concentration of 2.8-4.1 ppm.

6. Ref. 2 (sodium bisulfite)

The activity of serine deaminase from rat liver was inhibited 20% by 6.7×10^3 sodium bisulfite.

7. Ref. 141 (sodium sulfite)

Vitamin B_{12_r} and methyl iodide in the presence of Na_2SO_3 form cobalamin sulfonate. In the presence of most other simple electrolytes (NaCl, KCl, Na_2SO_4 or $MgCl_2$) methylcobalamin is formed from vitamin B_{12_r} and methyl iodide.

8. Ref. 88 (sodium bisulfite and sulfur dioxide)

$NaHSO_3$ caused a small decrease in the viscosity of blended egg whites. The concentration of thioglycol necessary to produce the same effect was only one twentieth that of $NaHSO_3$, however. Significant damage was also cause to whole eggs stored in an atmosphere containing 0.1% SO_2 at 2°C for relatively short times (< 17 days). These effects were allegedly caused by reduction of S-S bonds of egg white.

9. Ref. 133 (potassium metabisulfite)

The oxidation of tyrosine and pyrogallol by Agaricus campestris, in the presence of various vitamins with antioxidants properties (thiamine, ascorbic acid and nicotinic acid), was studied. The addition of small quantities of potassium metabisulfite had a very marked synergistic effect upon the antioxidant properties of these vitamins and, in addition, inhibited the formation in this system of thiochrome, an oxidation product of thiamine.

10. Ref. 30 (sodium sulfite)

The disulfite bonds of ox insulin were found to react with Na_2SO_3 over the range, pH 2-9, to form RS^- and $RS\text{-}SO_3^-$. Only two of the three disulfide bonds were reactive in the presence of $Na_2 \cdot SO_3$ alone, however, when 6M urea or excess phenylmercuric hydroxide were added, the third disulfide bond was found to be reactive. The first two disulfide bonds linked chains A and B of the molecule and the third was an intrachain disulfide bond on chain A.

11. Ref. 75 (sulfur dioxide)

Oral administration, twice in four hours, of 5ml of an aqueous solution containing 50 mg/l SO_2 leads to a 100% increase in the level of C-17-keto-steroids in the urine of male rats by day 2. Administration of a 12% ethanol solution in 50 mg/l SO_2 led to a 25% hormonal increase by day 2. Since white wine, containing both ethanol and sulfurous acid, leads to a highly significant C-17-ketosteroid output, there must be other factors, complementary to the SO_2 effect, responsible for hormone release.

12. Ref. 139 (sodium sulfite)

Sulfurase, isolated from bakers' yeast, catalyzed the formation of APS and pyrophosphate from ATP and also the exchange of radioactive pyrophosphate with ATP in the presence of sulfate, its normal substrate. In the presence of sulfite only AMP and pyrophosphate were formed and no exchange of PP with ATP was observed.

13. Ref. 100 (sulfur dioxide)

Exposure of mature male rabbits to 3 ppm SO_2 in air for 20 min. daily for 5 weeks led to no change in body wt., blood count, Hb, or hematocrit. With 5 ppm the above indexes show initial increases followed by rapid recovery; with greater than 10 ppm, there were decreases followed by slow recovery.

14. Ref. 28 (sodium bisulfite and sulfite)

Bovine prothrombin and autoprothrombin C showed reduced clotting function after being treated with Na_2SO_3 or $NaHSO_3$ for 2 hours. A 24 hour treatment was required with each of these reagents before a marked inhibitory affect in bovine thrombin activity could be observed.

15. Ref. 131 (sulfur dioxide)

White male rats (250 gm average weight) developed blood acidosis after application of 0.27 mg/1 SO_2 during a 4 hour period. There appeared a drop in blood pH from 7.333 to 7.291, a drop in blood CO_2 and bicarbonate and a decrease in buffering bases. The urine contained an increased amount of acids and ammonium ion. Conditions had not yet returned to normal after three days.

16. Ref. 76 (sodium bisulfite)

A p.o. dose of 12.5 mg/kg $NaHSO_3$ in a rabbit induced a maximum 10% hyperglycemia within 30 minutes of feeding, with a subsequent fall to a 5% hypoglycemia 2 1/2 hours later and normalization 4 1/2 hours post-treatment. A 20, 35 and 30% hyperglycemia followed p.o. administration in 3 separate rabbits of 24, 26 and 37 mg/kg, resp., in 30 minutes. The 20% rise in blood sugar was followed by a dramatic 30% fall (including a 10% hypoglycemia) within the next 3 hours and normalization by 4 1/2 hours post-treatment. The slope of the fall in sugar level after the 35 and 30% hyperglycemia was less severe, with no hypoglycemia and a return to normal 4 1/2 hours post-treatment. Subcutaneous administration (1.2, 2.4 and 4.8 mg/kg) elicited similar curves but with less acute initial slopes; 20% hyperglycemic maxima were reached

by 1 hour post-treatment with normalization by 4 1/2 hours.

17. Ref. 55 (sulfur dioxide, sodium sulfite and bisulfite)

Rapid jets of SO_2 were applied to vital rabbit cornea for 5 seconds and the response followed for up to 6 months. In moist buffered tissue the SO_2 is converted mainly to "H_2SO_3". The rise in hydrated SO_2 immediately post-treatment confirmed the facile permeability in cornea and conjunctiva; the concentration of SO_2 in all forms (H_2SO_3, acid bisulfites or neutral sulfites) in cornea fell exponentially, with a 15 minute half-life. Since H_2SO_4 was not increased in corneal extracts, it was inferred that SO_2 was not significantly oxidized to sulfuric acid. Moreover, biochemical assay of corneal extracts showed markedly diminished catalase. Ths histology found conjunctival chemosis and injection with greatly thickened corneal stroma and epithelium. Only 6 months post-treatment was there obvious evidence of clearing and sufficient vascularization. The lesions were typical of severe acid burns, with the exception of a striking eosinophilia in corenal infiltrate and no insult to corneal endothelium.

Irrigation of vital eye by neutral 1.4M sodium sulfite (an amount equivalent to 2.15 mg SO_2) led only to minor injection and chemosis, with no SO_2- type opacity and complete normalization within 2 days. Irrigation by 5.5M sodium bisulfite at pH 3.8 (an amount equivalent to 4.63 mg SO_2) induced intense conjunctival chemosis and injection, epithelial loss and opacity. The pathology cleared in 10 days without vascularization.

"Sulfurous acid" was the most cornea-permeable acid at both pH 1 and pH 2 and was 4x more rapid than sulfuric acid, a property not attributable to dissociation alone.

18. Ref. 11 (sulfur dioxide)

A single dose of aqueous SO_2 dose was given i.v. in 10 rabbits with sacrifice at 1 hour post-treatment. Manometric assay of blood after 1 hour incubation with 0.3 M cysteine found erythrocyte oxygen consumption reduced by ca. 18%. Erythrocyte decarboxylation was also only 85% of controls. Glyoxal assay found reduced

glutathione decreased by 50% in liver and lung, 40% in kidney, but within normal range in adrenal, spleen and blood.

19. Ref. 12 (sulfur dioxide)

A single i.p. dose of 5mg aqueous SO_2; two doses of $5mgSO_2$ and three doses of $10mgSO_2$ were administered to 3 groups of guinea pigs. Electrophoretic assay of heparinized blood was made after decapitation. There was no change in reduced or total serum glutathione levels after the single injection. After 2 injections the reduced gluthione increased 132%, and the total glutathione 154%, over controls. However, the increase in both glutathione components after 3 injections was only 113%.

20. Ref. 31 (sulfur dioxide)

A semi-quantitative "leuko-ascorbic acid" index has been proposed as a measure of leukocyte response to toxic exposure. Based on the intensity of ascorbic acid staining in eosinophils, monocytes, lymphocytes and neutrophils, in order of intensity, the index is equivalent to $(0 \cdot A+1 \cdot B+2 \cdot C+3 \cdot D)/100$ where A, B, C, D represent the number of cells with negative or weakly, moderately and strongly positive reactions. The values 0, 1, 2, 3 are coefficients only.

The normal index is 1.38 (0.99-1.52). In 9 cases of occupational SO_2 intoxication the index was 1.6 (1.5-1.7), with 40-55% of all cell types in moderate staining range, 28-42% in the weak range and 6-15% in the intense range. Increased ascorbic acid in circulating leukocytes is characteristic of SO_2 exposure, while silicosis, cyanide poisoning or saturnism exhibit decreases.

21. Ref. 126 (sodium bisulfite)

At a conc of 0.15M sodium bisulfite was reacted with $.5-7x10^3$ units of a histidine deaminase from *Pseudomonas* sp. (ATTC 11299b), spec. act. of $4.15x10^5$ units/mg. After 2500-fold dilution and assay of enzyme activity, complete inhibition was the result. The mechanism is thought to involve reaction with a carbonyl group of the enzyme. Other carbonyl reagents also are inhibitory.

22. Ref. 123 (sodium bisulfite)

RNA was separated chromatographically from the reaction between 5mg sodium yeast ribonucleate and 118mg sodium bisulfite at pH 5. Subsequent fractionation and spectrophotometry recovered all of the adenylic and guanylic acid. However, there was an initial time-dependent 45-92% deamination of cytosine residues to uracil; the reaction rate finally levelled off after ca. 90% cytosine deamination. The reactivity of cytosine-bisulfite is thought to be strongly influenced by the secondary structure of RNA.

23. Ref. 130 (sulfur dioxide)

Mouse L-strain fibroblasts (NCTC 929), mouse parenchymal liver cells (NCTC 1769) and human HeLa cultures were flushed with graded concentrations of SO_2 gas for 30 seconds every 48 hours for 12 days.

In medium A, consisting of 20% chick embryo extract and 40% horse serum, 500 ppm SO_2 had no effect on fibroblast or parenchymal cell proliferation and even potentiated HeLa growth, especially after day 6. At 1000 ppm significant fibroblast and HeLa loss began at the sixth day and reached 40% and 50%, respectively, by the twelfth. Liver parenchyma was less sensitive until the eighth day when the effect accelerated to 60% by day 12. Fibroblast inhibition by 2000 ppm was 84% and 91% by days 6 and 12, respectively. Parenchymal cells were again less sensitive, with an LD_{50} at the sixth and LD_{72} at the twelfth day. HeLa cells lost 73% and 85%, respectively, by days 6 and 12.

A similar inhibition was obtained with 10x lower SO_2 concentrations when the cell systems were grown in semisynthetic medium with 10% horse serum and a reduced buffering potential (acid shift to pH 6.8). Serum prophylaxis is evident, suggesting combination between the gas and same serum component.

Fibroblast monolayers in semisynthetic medium were also exposed to three different SO_2 concentrations for eight hours followed by a 16 hour recovery. Growth in those test cultures with a 40x higher death rate was about 61% that of the controls at 25 ppm SO_2. At

10 ppm, test culture growth was 80% of the controls and the number of non-viable cells 12x higher; and at 5 ppm the number of non-viable cells was 11x greater, although test culture growth was 96% of the controls. Various subsequent extensions of the SO_2 exposure interval at the same three concentrations confirmed the pattern of reduced proliferation and increased death rate, both of which variables were dose- and time-dependent. In all cases the majority of detached cells in the experimental cultures were non-viable, in contrast to the controls, even though the number of those test cells may not have significantly exceeded the control number. The maximum response (LD_{100}) followed eight hours exposure to 25 ppm on two successive days.

24. Ref. 60

Sulfiting of vegetables was reported to reduce the severity of meteorism in rats.

25. Ref. 21(sodium metabisulfite)

Agar gel electrophoresis of whole beef brain extracts was performed with and without 1.66×10^{-4} M sodium metabisulfite. As with sodium sulfite inhibition, there was a differential inhibition of 3 isozyme components varying between 40 and 70%. Native LDH was 84% suppressed by metabisulfite.

At the same concentration there was no spectrophotometric evidence of reaction between metabisulfite and DPN or DPNH. However, an instantaneous complex was formed with 0.38 M metabisulfite and 1 microM DPN at pH 7.4. Metabisulfite attack on DPN was not sensitive to pH changes, in contrast to sulfite-DPN complexes, ostensibly confirming that $S_2O_5^{=}$ exists as such in aqueous solution. Because metabisulfite is a competitive inhibitor and the LDH isozyme fractions (reflecting conformational changes during migration) manifest degrees of inhibition, it is assumed that the anion inhibits the active site or some near neighbor

V. Drug Interaction

1. Ref. 143 (sodium metabisulfite)

A 10^{-3}M concentration of sodium metabisulfite inhibited serotonin degradation by ceruloplasmin in vitro at pH 7.4 by ca. 60%. The effect is similar to that of ascorbic acid, glutathione and other reducing agents.

2. Ref. 22 (sodium bisulfite)

The antibacterial action of streptomycin toward *E. Coli* is antagonized by various reducing agents, among them, sodium bisulfite. The authors speculate only vaguely on a possible mechanism.

3. Ref. 125 (sodium bisulfite)

A combined sodium bisulfite-benzoic acid regimen (80-40 mg/kg, resp.) for 3.5 months in mice between weaning and mating led to the following effects on weight gain or loss in successive generations:

Sex	F_1		F_2		F_3		F_4	
	M	F	M	F	M	F	M	F
% of weight at weaning	-32%	-27%	-30%	-16%	-20%	+2%	+60%	+160%

After 17 months of the same combined diet as above, 8/100 F_1 and 1/8 F_3 mice in the same group were found to have malignant tumors.

Between 35 and 44% of the males and 29-55% of the females had survived for 8 months on the combined regimen.

The weight loss for male and female rats, resp, was 20 and 10% after 18 months on the combined sulfite-benzoic acid supplement cited above, levels far below those for a sorbic acid-nisin dietary mix. Serum complement titer and leukocyte phagocytic activity were also higher in the latter. Serum ceruloplasmin and erythrocyte sedimentation rate was higher and sensitivity to

centrifugation (rotation rate of 90-300 revs/min for 15-30 min), cold stress (-2- +8°C for 12-24 hrs at end of 18 month treatment) and kidney loading with K_2HPO_4 was the greater in the bisulfite-benzoic acid combination. Blood ketone level was 21% lower than controls and 98% below a sorbic acid-nisin combination. Blood alkalinity, C-reactive protein and hematology were normal. CCl_4 (0.6 ml) given at the end of 18 months of a bisulfite-benzoic acid diet caused an LD_{60}, while a 27% mortality followed 0.2 ml daily in diet for 15 days in the same group of rats. A single shocking dose of 3400 mg/kg of sodium benzoate led to an LD_{100} at the end of 18 months of a sodium bisulfite-benzoic acid regimen. The evidence would suggest that the toxicity of bisulfite and benzoic acid separately is potentiated in combination.

4. Ref. 9 (sulfur dioxide)

Inhalation of SO_2 by 16 male rabbits exposed to 0.02 ppm for 113 days at 7 hours daily was affected concurrently with i.v. immunization by type XIX penumococcus (0.5 ml per animal for 6 periods of 3 consecutive daily doses at 5 day intervals, followed by a single 0.5 ml booster 60 days post-treatment). Blood was sampled 5 days after the end of vaccination on day 48 and after the booster on day 60.

It was found that prolonged exposure to low SO_2 concentrations inhibits antipneumococcal antibodies, with an exponential and control animals. Agglutinins rose sharply in the test group during the first week and much less so from day 8 to day 48, the end of immunization. Subsequent decline was scarcely reversed by the booster. Control slopes rose less sharply but reached much higher levels, such that the difference between experimentals and controls at the end of immunization was highly significant. The booster response was also highly significant. A rise in complement began only after 2 weeks in the test animals and after 3

weeks in the controls. The fall in complement began only on day 24 and continued sharply with only negligible recovery after the booster in the test group. The control curve fell much less sharply, after reaching a maximum on day 32, with a marked recovery after the booster.

5. Ref. 138 (sodium bisulfite)

Dietary sodium bisulfite at 1600 mg/kg levels in feeding studies on rats with thiamine supplementation were followed for 8-12 weeks. Low thiamine concentrations of 0.2 mg per 100 g feed led to an LD_{100} bisulfite intoxication. At 0.5 mg thiamine all animals survived but the weight increase was significantly below that for 1.0 or 2.0 mg levels. At the latter doses there was no difference in weight gain over controls. It is concluded that SO_2 toxicity can be minimized by thiamine levels above those normally required. However, a 5-fold or greater thiamine increase over the normal requirement does not raise the toxic threshold of SO_2.

Parenterally, the SO_2 toxicity threshold was unaffected by thiamine dose. Neither were thiamine-deficient animals more sensitive to SO_2 nor were animals receiving a dose 5-fold the normal vitamin requirement more resistant.

There was a 35% Hb loss at 0.2 mg, 40% at 1.0 mg and 45-50% at 0.1 mg thiamine s.c., with bisulfite diets equivalent to 2000 mg/kg SO_2. Administration of more than 800 mg/kg SO_2 led to depressed Hb levels, independent of thiamine dose. The mechanism is unknown.

VI. Consumer Exposure Information

The results of the NAS/NRC questionnaire are presently unavailable. In lieu of that, the following table (Lishmund, R.E.J. 1969. Sorbic Acid. Food Proc. Ind. 38: 51-53) is presented:

TABLE II
Per Head
Potential intake of sulphur dioxide in mg/day

Food	oz/wk	g/day	ppm	mg/day
Beer	90·3	365·7	70	25·6
Cabbage, dehydrated			2 500	
Candied peel, etc.			100	
Cider	2·56	10·4	200	2·1
Coffee extract, solid	0·30	1·2	150	0·23
Flavouring emulsions			350	
Flavouring syrups			350	
Flour/biscuit manufacture			200	
Fruit crystallised/glace			100	
Fruit dried	1·03	4·2	2 000	8·4
Fruit/Fruit pulp/manufacturing	0·59	2·4	3 000	7·2
Fruit juices/Welfare orange juice	0·54	2·2	350	0·8
Fruit—other			350	
Gelatin			1 000	
Ginger, dry root			150	
Sacramental grape juice			70	
Horse radish fresh grated			100	
Jam	2·96	12·0	100	1·2
Pectin liquid			250	
Perry			200	
Pickles & Sauces	1·21	5·0	100	0·5
Potatoes raw peeled			50	
Potatoes dehydrated			550	
Sausages or sausage meat	3·72	15·1	450	6·8
Soft drinks (calc. as all diluted)	30·8	124·7	70	8·7
Starches, prepared			100	
Starch, hydrolysed (solid)			70	
Starch, hydrolysed (syrup)			450	
Sugar or sugar syrups	17·68	71·60	70	5·1
Tomato pulp, paste or puree			350	
Vegetable dehydrated (other than cabbage or potato			2 000	
Vinegar			70	
Wine	3·9	15·8	450	7·1
TOTAL				73·73

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